

SANTA ANA WATERSHED PROJECT AUTHORITY



Quality Assurance Project Plan

February 2016



**CDM
Smith**

Santa Ana River Watershed Bacteria Monitoring Program

Quality Assurance Project Plan

Prepared by

CDM Smith

On Behalf of

Santa Ana Watershed Project Authority

Version 1.0

February 2016

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Group A: Project Management

1. Title and Approval Sheet

Table 1-1. Quality Assurance Project Plan (QAPP) approval sheet (Table 1-1 shall be updated as needed to remain current)

Role ¹	Position ²	Agency/Entity ³	Name	Signature	Date
Monitoring Plan & QAPP Regulatory Oversight	Project Director	Santa Ana Watershed Project Authority	Rick Whetsel		
Monitoring Plan & QAPP Regulatory Oversight	Project Manager	Santa Ana Regional Water Quality Control Board	Ken Theisen		
Monitoring Plan & QAPP Regulatory Oversight	QA Officer	State Water Resources Control Board	Renee Spears		
Responsible Agency	Project Manager/ Project QA Officer	Agriculture/Dairy Representative	Pat Boldt		
Responsible Agency	Project Manager	City of Claremont	Loretta Mustafa		
Responsible Agency	Project QA Officer	City of Claremont	Kimberly Colbert		
Responsible Agency	Project Manager	City of Pomona	Julie Carver		
Responsible Agency	Project QA Officer	City of Pomona	Rae Beimer		
Responsible Agency	Project Manager	Orange County Watersheds (Orange County Public Works)	Jian Peng		
Responsible Agency	Project QA Officer	Orange County Watersheds (Orange County Public Works)	Kurt Zach		
Responsible Agency	Project Manager/ Project QA Officer	Riverside County Flood Control & Water Conservation District	Stuart McKibbin		

Table 1-1. Quality Assurance Project Plan (QAPP) approval sheet (Table 1-1 shall be updated as needed to remain current)

Role ¹	Position ²	Agency/Entity ³	Name	Signature	Date
Responsible Agency	Project Manager/ Project QA Officer	San Bernardino County Flood Control District	Marc Rodabaugh		
Responsible Agency (Designee)	Project Manager	CDM Smith	Richard Meyerhoff		
Responsible Agency (Designee)	Project QA Officer	CDM Smith	Barbara Wells		
Responsible Agency (Designee)	Project Manager	CWE	Vik Bapna		
Responsible Agency (Designee)	Project QA Officer	CWE	Gerald Greene		
Contract Laboratory	Laboratory Manager/Director/ QA Officer	Orange County Public Health Laboratory	Joseph Guzman		
Contract Laboratory	Laboratory Manager/Director	Orange County Water District	Donald Phipps		
Contract Laboratory	Laboratory QA Officer	Orange County Water District	Menu Leddy		
Contract Laboratory	Laboratory Manager/Director	Enthalpy Analytical	Cam Phan		
Contract Laboratory	Laboratory QA Officer	Enthalpy Analytical	Cliff Baldrige		
Contract Laboratory	Laboratory Manager/Director	Weck Laboratories	Kim Tu		
Contract Laboratory	Laboratory QA Officer	Weck Laboratories	Alan Ching		
Contract Laboratory	Laboratory Manager/Director	Weston Solutions Laboratory	Alex Schriewer		

Table 1-1. Quality Assurance Project Plan (QAPP) approval sheet (Table 1-1 shall be updated as needed to remain current)

Role ¹	Position ²	Agency/Entity ³	Name	Signature	Date
Contract Laboratory	Laboratory QA Officer	Weston Solutions Laboratory	Satomi Yonemasu		
Contract Laboratory	Laboratory Manager/Director	Babcock Laboratories, Inc.	Amanda Porter		
Contract Laboratory	Laboratory QA Officer	Babcock Laboratories, Inc.	Stacey Fry		
Contract Laboratory	Laboratory Manager/Director	Clinical Laboratory of San Bernardino, Inc.	Bob Glaubig		
Contract Laboratory	Laboratory QA Officer	Clinical Laboratory of San Bernardino, Inc.	Roberto Cabrera		
Contract Laboratory	Laboratory Manager/Director	Source Molecular	Mauricio Larenas		
Contract Laboratory	Laboratory QA Officer	Source Molecular	Tania Madi		

¹ **Role:** Monitoring Plan & QAPP Regulatory Oversight; Responsible Agency (or designee); or Contract Laboratory

² **Position:** For example, Project Director, Santa Ana Water Board, Project Manager, Project QA Officer, Monitoring Manager, Data Manager, Sampling Personnel, Laboratory Personnel

³ **Agency/Entity:** Name of the organization where the signatory is employed

⁴ **Title:** Job title within the organization that the signatory is employed

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- E. Weck Laboratories, Inc., Standard Operating Procedures
- F. Enthalpy Analytical (formerly Associated Laboratories), Standard Operating Procedures
- G. Weston Solutions Laboratory, Standard Operating Procedures
- H. Source Molecular, Standard Operating Procedures

Acronym List

AgSEP	Agricultural Source Evaluation Plan
AgSEMP	Agricultural Source Evaluation Monitoring Program
Basin Plan	Water Quality Control Plan for the Santa Ana River Basin
BASMP	Bacteria Indicator Agriculture Source Evaluation Plan
BMP	best management practice
BPA	Basin Plan amendment
°C	degrees Celsius
CBRP	Comprehensive Bacteria Reduction Plan
CEDEN	California Environmental Data Exchange Network
cfs	cubic feet per second
cfu	colony forming unit
COC	chain of custody
DPD	N,N-Diethylparaphenylenediamine
<i>E. coli</i>	<i>Escherichia coli</i>
EPA	Environmental Protection Agency
ft	feet
Los Angeles Water Board	Los Angeles Regional Water Quality Control Board
MBAS	Methylene blue active substances
mL	milliliters
mg/L	Milligram/liter
mg/mL	milligrams/milliliter
MP	Monitoring Plan
MPN	most probable number
mS/cm	millisiemens/centimeter
MS4	Municipal Separate Storm Sewer System
MSAR	Middle Santa Ana River
MSAR Bacteria TMDL	MSAR Bacterial Indicator TMDL
MSAR TMDL Task Force	MSAR Watershed TMDL Task Force
NTU	Nephelometric Turbidity Unit
OCC	Orange County Coastkeeper
OCWD	Orange County Water District
PPE	Personal protection equipment
Project ID	Project Identification Number
Q	flow
QA	quality assurance
QA/QC	quality assurance/quality control

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QAPP	Quality Assurance Project Plan
qPCR	
REC1	water contact recreation
REC2	non-contact water recreation
RMP	Regional Monitoring Program
Sample ID	Sample Identification Number
SAR Bacteria Monitoring Plan	Santa Ana River Watershed Bacteria Monitoring Plan
SAR Bacteria Monitoring Program	Santa Ana River Watershed Bacteria Monitoring Program
Santa Ana Water Board	Santa Ana Regional Water Quality Control Board
SAR	Santa Ana River
SAWPA	Santa Ana Watershed Protection Authority
Site ID	Site Identification Number
SM	Standard Method
State Water Board	State Water Resources Control Board
SWAMP	Surface Water Ambient Monitoring Program
t	Time (seconds)
TMDL	Total Maximum Daily Load
TSS	total suspended solids
UAA	use attainability analysis
USEP	Urban Source Evaluation Plan

3. Distribution List

Table 3-1 identifies the entities that shall receive a copy of a final approved QAPP. These same entities shall participate in revisions to this document. It will be up to each entity or agency to distribute copies of the QAPP within their organizations, where needed.

Table 3-1. QAPP distribution list¹

Role	Entity/Agency	Position	Contact Name, Tel. No., Email	QAPP Code
Monitoring Plan & QAPP Oversight	Santa Ana Watershed Project Authority (SAWPA)	Project Director	Rick Whetsel, 951-354-4222, rwhetsel@sawpa.org	SAR BACT Monitoring
	Santa Ana Regional Water Quality Control Board (Santa Ana Water Board)	Project Manager	Ken Theisen, 951-320-2028, ktheisen@waterboards.ca.gov	SAR BACT Monitoring
	State Water Resources Control Board	QA Officer	Renee Spears, 916-341-5583, renee.spears@waterboards.ca.gov	SAR BACT Monitoring
Responsible Agencies or Designee	Agriculture/Dairy Representative	Project Manager	Pat Boldt, 951-808-8631, mpboldt@aol.com	SAR BACT Monitoring
		Project QA Officer		SAR BACT Monitoring
	City of Claremont	Project Manager	Loretta Mustafa, 909-399-5475, lmustafa@ci.claremont.ca.us	SAR BACT Monitoring
		Project QA Officer	Kimberly Colbert, 310-729-8031, Kimberly@ColbertGroup.com	SAR BACT Monitoring
	City of Pomona	Project Manager	Julie Carver, 909-620-3628, Julie_carver@ci.pomona.ca.us	SAR BACT Monitoring
		Project QA Officer	Rae Beimer, 714-788-6936, raebeimer@caaprofessionals.com	SAR BACT Monitoring
	Orange County Watersheds (Orange County Public Works)	Project Manager ²	Jian Peng, 714-955-0650, jian.peng@ocpw.ocgov.com	SAR BACT Monitoring
		Project QA Officer	Kurt Zach, 714-955-0681, kurt.zach@ocpw.ocgov.com	SAR BACT Monitoring
	Riverside County Flood Control & Water Conservation District	Project Manager ²	Stuart McKibbin, 951-955-1273, smckibbi@rcflood.org	SAR BACT Monitoring
		Project QA Officer		SAR BACT Monitoring
	San Bernardino County Flood Control District	Project Manager ²	Marc Rodabaugh, 909-387-8112, marc.rodabaugh@dpw.sbcounty.gov	SAR BACT Monitoring
		Project QA Officer		SAR BACT Monitoring
	CDM Smith (Designee)	Project Manager ²	Richard Meyerhoff, 303-345-3083, meyerhoffrd@cdmsmith.com	SAR BACT Monitoring
		Project QA Officer	Barbara Wells, 816-412-3112, wellsbl@cdmsmith.com	SAR BACT Monitoring
	CWE (Designee)	Project Manager ²	Vik Bapna, 714-526-7500, VBapna@cwecorp.com	SAR BACT Monitoring
		Project QA Officer	Gerald Greene, 714-526-7500, GGreene@cwecorp.com	SAR BACT Monitoring
Contract Laboratory	Orange County Public Health Laboratory	Laboratory Manager/Director ³	Joseph Guzman, 949-219-0424, jguzman@ocha.com	SAR BACT Monitoring
		Laboratory QA Officer		SAR BACT Monitoring

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Table 3-1. QAPP distribution list¹

Role	Entity/Agency	Position	Contact Name, Tel. No., Email	QAPP Code
	Orange County Water District	Laboratory Manager/Director ³	Donald Phipps, 714-378-3200, dphipps@ocwd.com	SAR BACT Monitoring
		Laboratory QA Officer	Menu Leddy, 714-378-3313, mleddy@ocwd.com	SAR BACT Monitoring
	Enthalpy Analytical (formerly Associated Laboratories)	Laboratory Manager/Director ³	Cam Pham, 714-771-6900, cam.pham@enthalpy.com	SAR BACT Monitoring
		Laboratory QA Officer	Cliff Baldridge, 714-771-6900, cliff.baldridge@enthalpy.com	SAR BACT Monitoring
Contract Laboratory	Weck Laboratories, Inc.	Laboratory Manager/Director ³	Kim Tu, 626-336-2139 x118, Kim.Tu@wecklabs.com	SAR BACT Monitoring
		Laboratory QA Officer	Alan Ching, 626-336-2139 x116, Alan.Ching@wecklabs.com	SAR BACT Monitoring
	Weston Solutions Laboratory	Laboratory Manager/Director ³	Alex Schriewer, 760-795-6957, Alexander.Schriewer@WestonSolutions.com	SAR BACT Monitoring
		Laboratory QA Officer	Satomi Yonemasu, 760-795-6900, satomi.yonemasu@westonsolutions.com	SAR BACT Monitoring
	Babcock Laboratories, Inc.	Laboratory Manager/Director ³	Amanda Porter, 951-653-3351, x249, aporter@babcocklabs.com	SAR BACT Monitoring
		Laboratory QA Officer	Stacey Fry, 951-653-3351, sfry@babcocklabs.com	SAR BACT Monitoring
	Clinical Laboratory of San Bernardino, Inc.	Laboratory Manager/Director ³	Bob Glaubig, 909-825-7693, glaubig@clinical-lab.com	SAR BACT Monitoring
		Laboratory QA Officer	Roberto Cabrera, 909-825-7693, cabrera@clinical-lab.com	SAR BACT Monitoring
	Source Molecular	Laboratory Manager/Director ³	Mauricio Larenas, 786-416-6010, mlarenas@sourcemolecular.com	SAR BACT Monitoring
		Laboratory QA Officer	Tania Madi, 786-220-0379, tmadi@sourcemolecular.com	SAR BACT Monitoring

¹ Table information will be periodically reviewed and updated to reflect program changes.

² Project Manager or Project QA Officer within a Responsible Agency or its designee is responsible for distributing QAPP to other project participants including the Monitoring and Data Managers and Sampling Personnel (see Figure 4-1).

³ Laboratory Manager is responsible for ensuring laboratory staff are provided a copy of the QAPP.

4. Project/Task Organization

4.1 Overview

Figure 4-1 provides the organizational structure for implementation of the Santa Ana River (SAR) Watershed Bacteria Monitoring Program (“SAR Bacteria Monitoring Program”). The SAR Watershed Bacteria Monitoring Plan (“SAR Bacteria Monitoring Plan”) will be implemented by a number of Responsible Agencies under the direction of a Project Director and the Santa Ana Water Board, which provide oversight of the SAR Bacteria Monitoring Plan and QAPP. Within each Responsible Agency specific positions are shown; however, each agency may combine positions if more efficient for implementation.

Figure 4-1 illustrates the organizational structure for implementation of the SAR Bacteria Monitoring Program. The following subsections describe the responsibilities associated with various roles and positions shown in Figure 4-1. While the Project Director and Responsible Agencies are ultimately responsible for collection of water quality data and preparation of annual reports to fulfill the requirements of the SAR Bacteria Monitoring Plan and QAPP, some of the specific roles and responsibilities described below may be fulfilled through the use of contractors.

4.2 Monitoring Plan and QAPP Oversight

Two positions have been established to provide oversight to implementation of the SAR Bacteria Monitoring Plan and QAPP.

4.2.1 Project Director

The Project Director for the SAR Bacteria Monitoring Program is SAWPA. Table 4-1 summarizes Project Director’s overall responsibilities. While SAWPA will manage the overall program, SAWPA may contract portions of the work assigned to the Project Director position, e.g., preparation of the Annual Report.

4.2.2 Santa Ana Water Board

The Santa Ana Water Board is responsible for providing regulatory guidance for the implementation of the SAR Bacteria Monitoring Program. Specifically, the Santa Ana Water Board shall provide guidance to the parties implementing the SAR Bacteria Monitoring Plan and QAPP with regards to the requirements of the 2012 adoption of the Basin Plan Amendment (BPA) to *Revise Recreation Standards for Inland Freshwaters in the Santa Ana Region* (see Section 5 additional information). Accordingly, the Santa Ana Water Board has a Project Manager assigned to oversee implementation and that Project Manager will work with the State Water Resources Control Board’s (State Water Board) QA Officer to ensure the program, as described, is consistent with California Surface Water Ambient Monitoring Program (SWAMP) requirements.

Following approval of the SAR Bacteria Monitoring Plan and QAPP, the Santa Ana Water Board Project Manager and QA Officer shall be responsible for approvals of subsequent modifications to the SAR Bacteria Monitoring Plan and/or QAPP. The process for modifications of these documents is discussed in Section 1.4 of the SAR Bacteria Monitoring Plan.

4.3 Responsible Agency

For the purposes of this QAPP a Responsible Agency is an agency that is responsible for the collection of water quality data from at least one priority monitoring site and/or collection of water quality data to fulfill additional monitoring requirements established by a Total Maximum Daily Load (TMDL). Implementation of the SAR Bacteria Monitoring Plan and QAPP shall be completed by the following Responsible Agencies:

- Agricultural/Dairy Representative
- City of Claremont
- City of Pomona
- Orange County Watersheds (Orange County Public Works)
- Riverside County Flood Control and Water Conservation District
- San Bernardino County Flood Control District
- Others, as needed¹

For the purposes of this QAPP, Table 4-2 identifies the Responsible Agencies for implementation of water quality data collection at Regional Monitoring Program (RMP) priority monitoring sites and water quality data collection to fulfill additional TMDL monitoring requirements. It should be noted that two priority one sites (SAR at MWD Crossing and SAR at Pedley Avenue) and three priority two sites (Mill-Creek [Prado Area], Chino Creek at Central Avenue and Prado Park Lake) are shown as the responsibility of multiple entities. The Responsible Agencies for these sites will work collaboratively with the Project Director to determine final responsibility for collection of samples from these sites (e.g., by one of the Responsible Agencies, the Project Director, or a designated contractor) and establish any necessary cost-sharing agreements.

Within each Responsible Agency, five key positions have been identified to fulfill the requirements of the SAR Bacteria Monitoring Plan: Project Manager, Project QA Officer, Monitoring Manager, Data Manager and Sampling Personnel. Table 4-1 describes the duties assigned to each of the positions identified within each Responsible Agency. Where appropriate, a Responsible Agency may choose to combine two or more positions into a single position, e.g., combining Data Manager and Monitoring Manager activities. The Project QA Officer within each Responsible Agency shall ensure that the Quality Assurance and Quality Control (QA/QC) procedures contained herein are implemented as required within their area of responsibility.

Table 3-1 identifies the key roles and positions within each of the Responsible Agencies and the contact information for that position.

¹ Two monitoring sites in Orange County are surrounded by private or state lands. The agency that will be responsible for sampling these sites is still being determined.

4.4 Contract Laboratory

The Responsible Agencies shall select contract laboratories that have the capabilities to meet the requirements of this QAPP. Table 4-1 describes the responsibilities of each contract laboratory. The Laboratory Manager of each contract laboratory will be responsible for ensuring that Laboratory Personnel implement the requirements of this QAPP.

QAPP Organization Chart

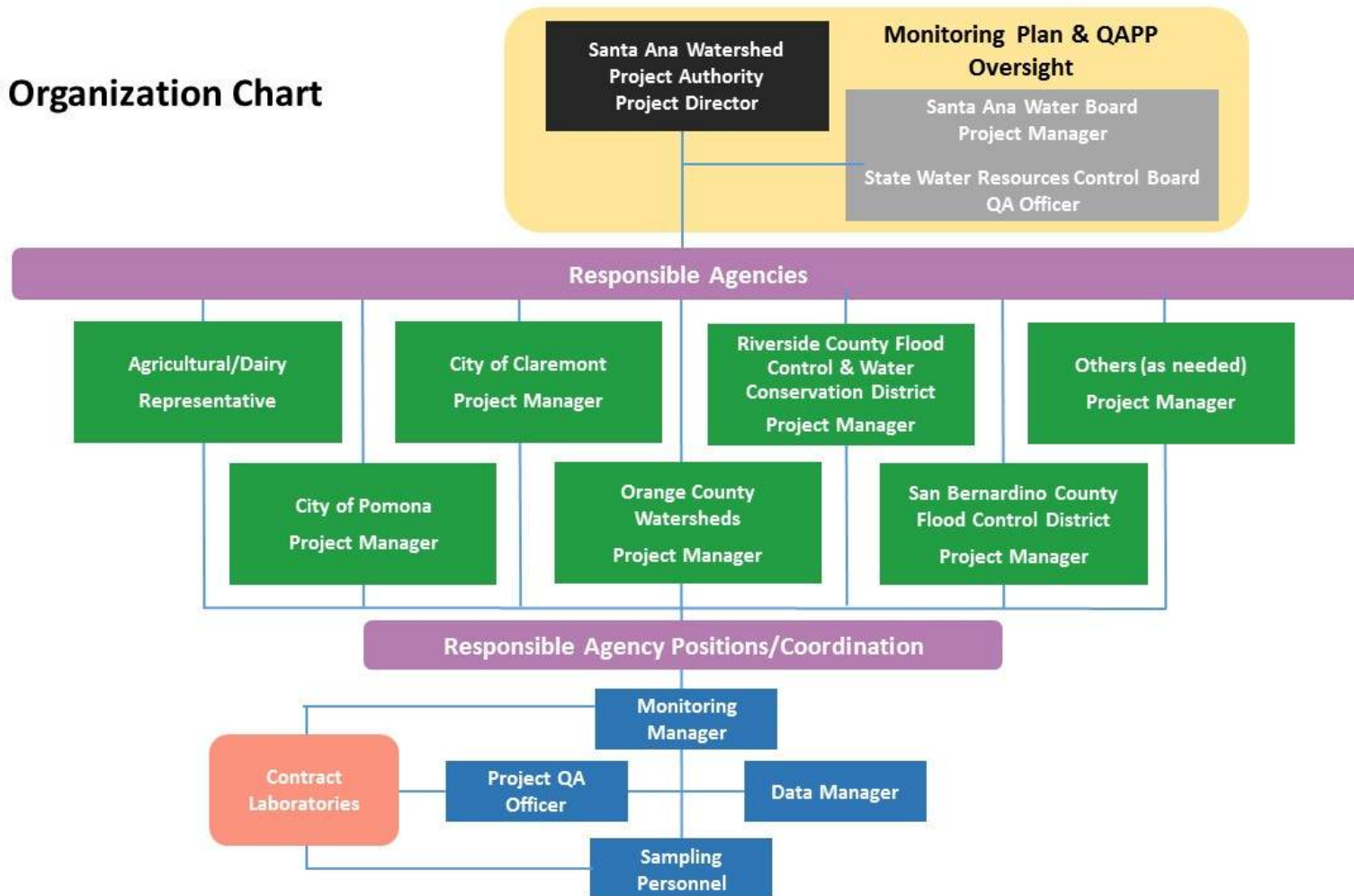


Figure 4-1. SAR Bacteria Monitoring Program organization chart

Table 4-1. Key positions & primary responsibilities for implementation of SAR Bacteria Monitoring Program

Position	Primary Responsibilities
Project Director	<ul style="list-style-type: none"> • Coordinate with Responsible Agencies to ensure that all monitoring sites shown in Table 4-2 are sampled annually as required by the Monitoring Plan and QAPP • Administration <ul style="list-style-type: none"> ○ Annual budget development and management ○ Establish/manage agreements/contracts, including cost share agreements where needed, taking into account which Responsible Agencies are collecting samples and where monitoring contracts need to be established ○ Coordination/communication with Regional Board, Counties, stakeholders • Reporting <ul style="list-style-type: none"> ○ Obtain all information/data results needed to prepare Annual Report ○ Complete data analyses as required ○ Prepare Annual Report; oversee review and revision process ○ Address data/information requests • Data Management <ul style="list-style-type: none"> ○ Provide database/spreadsheet template to Responsible Agencies for data entry during a sample year ○ Receive data transfers from Responsible Agencies ○ Conduct final data QA/QC ○ Annually upload dataset from previous sample year to California Environmental Data Exchange Network (CEDEN) • Oversee updates to the SAR Bacteria Monitoring Plan and QAPP, when required, and ensure appropriate approvals are obtained by Santa Ana Water Board
Santa Ana Water Board	<ul style="list-style-type: none"> • Provide guidance to the Project Manager and program management regarding the implementation of the SAR Bacteria Monitoring Plan and QAPP and the requirements set forth in the BPA to <i>Revise Recreation Standards for Inland Freshwaters in the Santa Ana Region</i> (approved by USEPA 2015) • Provide guidance to the Project Manager and program management with regards to California SWAMP requirements • Review and approval of revisions to originally approved SAR Bacteria Monitoring Plan and QAPP
Responsible Agency Project Manager	<ul style="list-style-type: none"> • Overall program management responsibility for the Responsible Agency • Coordinate with Project Director • Manage work of key positions within the Responsible Agency - either with in-house personnel or a contractor • Establish/manage contracts, as needed, to support monitoring activities • Invoice payment (e.g., contract laboratories/sampling, where contractors used) • Ensure sampling schedule is met (see Tables 10-1 and 10-2) • Ensure sufficient Sampling Personnel resources available • Address/solve programmatic issues as they arise • Manage project files while samples collected during a sample year • Ensure data uploaded into database/spreadsheet template provided by Project Director; at the end of a sample year, transmit all relevant project files/data to the Project Director • Coordinate with Project QA Officer; address/solve QA/QC issues as they arise • Make sure staff are properly trained

Table 4-1. Key positions & primary responsibilities for implementation of SAR Bacteria Monitoring Program

Position	Primary Responsibilities
Responsible Agency Project QA Officer	<ul style="list-style-type: none"> • Verify that the QA/QC procedures contained herein are implemented as required within their area of responsibility. • Verify QA/QC activities completed on data collected by a Responsible Agency before it is submitted to the Project Director and used to support development of the Annual Report. • The QA Officer may stop all actions, including those conducted by any subcontractor if there are significant deviations from required practices or if there is evidence of a systematic failure. Coordinate with Project Manager, Santa Ana Water Board and Project Director, as needed.
Responsible Agency Monitoring Manager	<ul style="list-style-type: none"> • Manage sampling personnel <ul style="list-style-type: none"> ○ Coordinate Sampling Personnel (in-house staff or contractors) to be sure sampling schedule for priority sites is met (see Tables 10-1 and 10-2) ○ If applicable – ensure TMDL-specific monitoring occurs as required, e.g., TMDL wet weather event ○ Conduct sample/equipment training as required ○ Ensure Sampling Personnel have all necessary equipment, forms, etc. to be successful • Manage equipment <ul style="list-style-type: none"> ○ Manage any equipment needs (e.g., flow meters, multi-parameter or similar instruments) ○ Ensure pre-field equipment procedures followed • Laboratory services <ul style="list-style-type: none"> ○ Coordinate sample collection with appropriate laboratories ○ If couriers used for sample delivery, coordinate courier scheduling ○ Ensure holding times are met ○ Ensure chain of custody (COC) forms properly managed • QA/QC <ul style="list-style-type: none"> ○ Coordinate with Project QA Officer to verify QA/QC procedures are followed, including equipment use, field blanks and replicates ○ Review field and COC forms for completeness ○ Work with Sampling Personnel, Contract Laboratories and Project QA Officer to resolve any issues of concern • Data Management <ul style="list-style-type: none"> ○ Receive data results from laboratories - verify completeness of results and conduct QA/QC check of laboratory results ○ Submit all field and laboratory documentation to Data Manager for data entry and filing
Responsible Agency Data Manager	<ul style="list-style-type: none"> • Enter field and laboratory data into database/spreadsheet template provided by Project Director • Conduct QA/QC of data entry process • Submit final dataset from a sample year to the Project Manager for transmittal to the Project Director

Table 4-1. Key positions & primary responsibilities for implementation of SAR Bacteria Monitoring Program

Position	Primary Responsibilities
Responsible Agency Sampling Personnel	<ul style="list-style-type: none"> • Trained in sample collection procedures and QAPP requirements • Complete field instrument pre- and post-sample collection calibrations • Collect field data and water sample as directed per the Monitoring Plan and QAPP • Complete COC forms and submit samples to the laboratory within holding times (including coordination with couriers if needed)
Contract Laboratories Laboratory Personnel	<ul style="list-style-type: none"> • Provide the necessary containers, preservatives (if required), COC forms to support sample collection • Analyze the samples for constituents as indicated in this QAPP and requested by the Monitoring Manager • Operate according to laboratory QA/QC program in accordance with guidelines established by the State of California and the U.S. Environmental Protection Agency (EPA) • Provide data in electronic and hard copy format to the Responsible Agency Monitoring Manager that submitted samples for analysis • Work with the Project QA Officers and Monitoring Manager for each Responsible Agency to resolve sample or data analysis issues when they arise

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Table 4-2. Responsible agencies for RMP priority sites and additional TMDL monitoring

Responsible Agency	Monitoring Type	Monitoring Sites
Agricultural & Dairy	Priority 1	<ul style="list-style-type: none"> SAR at MWD Crossing (also Priority 2) SAR at Pedley Avenue (also Priority 2)
	Priority 2	<ul style="list-style-type: none"> Chino Creek at Central Avenue Mill Creek (Prado Area) Prado Park Lake
	Priority 3	None
	Priority 4	None
	TMDL Specific	<ul style="list-style-type: none"> Middle Santa Ana River Bacteria TMDL – Wet Weather Event
City of Claremont	Priority 1	<ul style="list-style-type: none"> SAR at MWD Crossing (also Priority 2) SAR at Pedley Avenue (also Priority 2)
	Priority 2	<ul style="list-style-type: none"> Chino Creek at Central Avenue Mill Creek (Prado Area) Prado Park Lake
	Priority 3	None
	Priority 4	None
	TMDL Specific	<ul style="list-style-type: none"> Middle Santa Ana River Bacteria TMDL – Wet Weather Event
City of Pomona	Priority 1	<ul style="list-style-type: none"> SAR at MWD Crossing (also Priority 2) SAR at Pedley Avenue (also Priority 2)
	Priority 2	<ul style="list-style-type: none"> Chino Creek at Central Avenue Mill Creek (Prado Area) Prado Park Lake
	Priority 3	None
	Priority 4	None
	TMDL Specific	<ul style="list-style-type: none"> Middle Santa Ana River Bacteria TMDL – Wet Weather Event sampling at
Orange County Watersheds (Orange County Public Works)	Priority 1	None
	Priority 2	None
	Priority 3	<ul style="list-style-type: none"> Bolsa Chica Channel Borrego Canyon Wash Buck Gully Creek Peters Canyon Wash San Diego Creek, Reach 1 San Diego Creek, Reach 2 Santa Ana River, Reach 2 Serrano Creek
	Priority 4	Santa Ana Delhi Channel
	TMDL Specific	None

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Table 4-2. Responsible agencies for RMP priority sites and additional TMDL monitoring

Responsible Agency	Monitoring Type	Monitoring Sites
Riverside County Flood Control & Water Conservation District	Priority 1	<ul style="list-style-type: none"> • Canyon Lake • Lake Elsinore • Perris Lake • SAR at MWD Crossing (also Priority 2) • SAR at Pedley Avenue (also Priority 2)
	Priority 2	<ul style="list-style-type: none"> • Chino Creek at Central Avenue • Mill Creek (Prado Area) • Prado Park Lake
	Priority 3	<ul style="list-style-type: none"> • Goldenstar Creek • Lake Fulmor
	Priority 4	<ul style="list-style-type: none"> • Temescal Creek Reaches 1a and 1b
	TMDL Specific	<ul style="list-style-type: none"> • Middle Santa Ana River Bacteria TMDL – Wet Weather Event
San Bernardino County Flood Control District	Priority 1	<ul style="list-style-type: none"> • SAR at MWD Crossing (also Priority 2) • SAR at Pedley Avenue (also Priority 2) • Big Bear Lake at Swim Beach • Mill Creek Reach 2 • Lytle Creek, North Fork
	Priority 2	<ul style="list-style-type: none"> • Chino Creek at Central Avenue • Mill Creek (Prado Area) • Prado Park Lake
	Priority 3	<ul style="list-style-type: none"> • SAR above S. Riverside Avenue Bridge
	Priority 4	Cucamonga Creek, Reach 1
	TMDL Specific	<ul style="list-style-type: none"> • Middle Santa Ana River Bacteria TMDL – Wet Weather Event
Others, as needed	Priority 1	None
	Priority 2	None
	Priority 3	<ul style="list-style-type: none"> • Los Trancos Creek • Morning Canyon Creek
	Priority 4	None
	TMDL Specific	None

5. Problem Definition/Background

Bacterial indicator monitoring is conducted in the Santa Ana River watershed for three key purposes:

- Fulfill the monitoring and surveillance requirements for the 2012 adopted BPA to Revise Recreation Standards for Inland Freshwaters in the Santa Ana Region;
- Conduct sampling to support implementation of the Middle Santa Ana River (MSAR) Bacterial Indicator TMDL ("MSAR Bacteria TMDL"); and
- Support any additional bacterial indicator monitoring that may be conducted in the watershed to support regional regulatory activities.

5.1 Regulatory Background

This QAPP supports the implementation of several regulatory related activities associated with the protection of recreational uses in the Santa Ana River Watershed. The following subsections describe these activities and their regulatory importance.

5.1.1 Basin Plan Amendment

On June 15, 2012, the Santa Ana Water Board adopted the BPA to Revise Recreation Standards for Inland Freshwaters in the Santa Ana Region². This BPA resulted in the following modifications to the Water Quality Control Plan for the Santa Ana River Basin (Basin Plan) for the Santa Ana region³:

- Addition of "Primary Contact Recreation" as an alternative name for the REC1 (water contact recreation) beneficial use;
- Addition of narrative text clarifying the nature of REC1 activities and the bacteria objectives established to protect these activities.
- Differentiation of inland surface REC1 waters on the basis of frequency of use and other characteristics for the purposes of assigning applicable single sample maximum values.
- Revision of REC1/REC2 (non-contact water recreation) designations for specific inland surface waters based on the results of completed Use Attainability Analyses.
- Revised water quality objectives to protect the REC1 use of inland freshwaters
- Identification of criteria for temporary suspension of recreation use designations and objectives (high flow suspension)

Santa Ana Water Board staff developed this BPA in collaboration with the Stormwater Quality Standards Task Force, comprised of representatives from various stakeholder interests, including SAWPA; the counties of Orange, Riverside, and San Bernardino; Orange County Coastkeeper; Inland

² Santa Ana Water Board Resolution: R8-2012-0001, June 15, 2012

³ Page 2 of Attachment 2 to the Santa Ana Water Board Resolution: R8-2012-0001, as approved on June 15, 2012 and corrected on February 12, 2013 and November 15, 2013.

Empire Waterkeeper; and EPA Region 9. The BPA was approved by the State Water Board on January 21, 2014⁴ and the Office of Administrative Law on July 2, 2014⁵. The EPA issued its findings by letter on April 8, 2015 and provided a letter of clarification on August 3, 2015.

The BPA requires establishment of a comprehensive monitoring program to support implementation of the changes to the Basin Plan⁶. This QAPP and its accompanying SAR Bacteria Monitoring Plan have been submitted to the Santa Ana Water Board for approval⁷.

5.1.2 Bacteria TMDLs

Currently, there is one bacteria TMDL adopted for freshwaters in the Santa Ana River Watershed: MSAR Bacteria TMDL, which became effective in May 2007. Following is a brief summary of the establishment of this TMDL.

In 1994 and 1998, because of exceedances of the fecal coliform objective established to protect the REC1 use, the Santa Ana Water Board added the following waterbodies in the MSAR watershed to the state 303(d) list of impaired waters:

- Santa Ana River, Reach 3 – Prado Dam to Mission Boulevard
- Chino Creek, Reach 1 – Santa Ana River confluence to beginning of hard lined channel south of Los Serranos Road
- Chino Creek, Reach 2 – Beginning of hard lined channel south of Los Serranos Road to confluence with San Antonio Creek
- Mill Creek (Prado Area) – Natural stream from Cucamonga Creek Reach 1 to Prado Basin
- Cucamonga Creek, Reach 1 – Confluence with Mill Creek to 23rd Street in City of Upland
- Prado Park Lake

The Santa Ana Water Board adopted the MSAR Bacteria TMDL in 2005⁸; it was subsequently approved by the EPA on May 16, 2007. The TMDL established compliance targets for both fecal coliform and (*Escherichia coli*) *E. coli*:

- Fecal coliform: 5-sample/30-day logarithmic mean less than 180 organisms/100 milliliters (mL) and not more than 10 percent of the samples exceed 360 organisms/100 mL for any 30-day period.
- *E. coli*: 5-sample/30-day logarithmic mean less than 113 organisms/100 mL and not more than 10 percent of the samples exceed 212 organisms/100 mL for any 30-day period.

⁴ State Water Board Resolution: 2014-0005, January 21, 2014

⁵ Office of Administrative Law: #2014-0520 -02 S; July 2, 2014

⁶ Page 76 of Attachment 2 to the Santa Ana Water Board Resolution: R8-2012-0001, as corrected

⁷ Submitted June 30, 2015.

⁸ Santa Ana Water Board Resolution: R8-2005-0001, August 26, 2005

Per the TMDL, the above compliance targets for fecal coliform become ineffective upon EPA approval of the BPA⁹.

To focus MSAR Bacteria TMDL implementation activities, stakeholders established the MSAR Watershed TMDL Task Force (MSAR TMDL Task Force) to coordinate TMDL implementation activities designed to manage or eliminate sources of bacterial indicators to waterbodies listed as impaired. The MSAR TMDL Task Force includes representation by key watershed stakeholders, e.g., urban stormwater dischargers, agricultural operators, and the Santa Ana Water Board.

The MSAR Bacteria TMDL required urban and agricultural dischargers to implement a watershed-wide bacterial indicator compliance monitoring program by November 2007¹⁰. Stakeholders worked collaboratively through the MSAR TMDL Task Force to develop this program and prepared a Monitoring Plan and QAPP for submittal to the Santa Ana Water Board. The MSAR TMDL Task Force implemented the monitoring program in July 2007 following Santa Ana Water Board approval of monitoring program documents¹¹. The Monitoring Plan and QAPP have been updated as needed since 2007 with the most recent update occurring in 2013.

The MSAR Bacteria TMDL also required the development and implementation of plans by urban and agricultural dischargers within six months of the TMDL effective date:

- *Urban Dischargers* – Municipal Separate Storm Sewer System (MS4) permittees in Riverside and San Bernardino Counties within the MSAR watershed were required to submit a bacterial indicator Urban Source Evaluation Plan (USEP) within six months of the TMDL effective date. The purpose of this program was to identify activities, operations, and processes in urban areas that contribute bacterial indicators to MSAR watershed waterbodies.

The USEP was submitted to the Santa Ana Water Board in November 2007 and approved April 18, 2008¹². The USEP was replaced by Comprehensive Bacteria Reduction Plans (CBRP) prepared by Riverside and San Bernardino MS4 permittees to fulfill 2010 MS4 Permit requirements applicable to urban dischargers subject to the MSAR Bacteria TMDL requirements. The Santa Ana Water Board approved the CBRPs for these counties on February 10, 2012¹³. To fulfill 2012 MS4 Permit requirements, additional CBRPs were completed by the Cities of Pomona and Claremont for the portions of their cities that are within the MSAR watershed and subject to MSAR Bacteria TMDL requirements. These CBRPs were approved by the Santa Ana Water Board on March 14, 2014¹⁴. All CBRPs completed by MS4 dischargers include monitoring activities that to date have been covered by the Monitoring Plan and QAPP prepared by the MSAR TMDL Task Force (see above).

- *Agricultural Dischargers* – Agricultural operators in the MSAR watershed were required to submit an Agricultural Source Evaluation Plan (AgSEP) within six months of the TMDL effective date. The

⁹ Page 3 of 15 of Attachment A to Santa Ana Water Board Resolution R8-2005-0001.

¹⁰ Page 6 of 15, Table 5-9y of Attachment A to Santa Ana Water Board Resolution R8-2005-0001

¹¹ Santa Ana Water Board Resolution: R8-2008-0044, April 18, 2008

¹² Santa Ana Water Board Resolution: R8-2008-0044, April 18, 2008

¹³ Santa Ana Water Board Resolutions: R8-2012-0015 (Riverside County MS4 Program; R8-2012-0016 (San Bernardino County MS4 Program)

¹⁴ Santa Ana Water Board Resolution: R8-2014-0030 (City of Claremont); R8-2014-0031 (City of Pomona)

purpose of the AgSEP was to identify activities, operations, and processes in agricultural areas that contribute bacterial indicators to MSAR watershed waterbodies. The AgSEP included monitoring activities that have been covered by the Monitoring Plan and QAPP prepared by the MSAR TMDL Task Force (see above).

The AgSEP was submitted to the Santa Ana Water Board in November 2007 and approved April 18, 2008¹⁵. Currently, a Bacterial Indicator Agricultural Source Management Plan (BASMP) is under development. Once completed and approved by the Santa Ana Water Board, the BASMP will replace the AgSEP.

This QAPP incorporates all existing MSAR Bacteria TMDL QAPP requirements as described above. Accordingly, upon execution of the RMP, this QAPP replaces the existing MSAR Bacteria TMDL QAPP.

5.1.3 Waters Impaired for Bacterial Indicators

The State Water Board periodically publishes a list of impaired waters for the State of California, which is prepared according to the requirements of the State Water Board's *Water Quality Control Policy for Developing California's Clean Water Act Section 303(d) List*¹⁶. Subject to EPA Region 9 approval, the most recently approved 303(d) List is contained within the State Water Board's 2010 Integrated Report¹⁷. The State Water Board's 2010 Integrated Report website provides an estimated date for development of a TMDL for each listed waterbody. Any bacteria-related monitoring activities conducted in these 303(d) listed waterbodies are covered by this QAPP and accompanying Monitoring Plan.

5.2 Watershed Description

The Santa Ana River watershed covers an area of approximately 2,650 square miles and includes portions of Orange, Riverside, and San Bernardino County, and a small portion of Los Angeles County (see Figure 2-1 in the SAR Bacteria Monitoring Plan). The mainstem Santa Ana River is the primary waterbody in the watershed. It flows in a generally southwest direction nearly 100 miles, from its headwaters to the Pacific Ocean. The watershed can be generally divided into three major geographic areas:

- *San Jacinto River and Temescal Creek Region* – This area covers much of the south central and southeastern portions of the watershed and is located mostly within Riverside County. The San Jacinto River drains an area of approximately 780 square miles to Canyon Lake and Lake Elsinore. Often flows from the upper San Jacinto River watershed are captured by Mystic Lake, which is a natural sump or hydrologic barrier to flows moving further downstream to Canyon Lake or Lake Elsinore. Downstream of Lake Elsinore, Temescal Creek carries surface flow, when it occurs, from below Lake Elsinore to its confluence with Prado Basin.
- *Santa Ana River above Prado Dam and Chino Basin Region* – This area includes much of the north central and northeastern portions of the watershed and is located mostly within San Bernardino

¹⁵ Santa Ana Water Board Resolution: R8-2008-0044, April 18, 2008

¹⁶ http://www.waterboards.ca.gov/water_issues/programs/tmdl/docs/ffed_303d_listingpolicy093004.pdf

¹⁷ Final EPA approval – October 11, 2011; list of impaired waters in California, by region:

http://www.waterboards.ca.gov/water_issues/programs/tmdl/2010state_ir_reports/category5_report.shtml

County. This region drains to Prado Basin where Prado Dam captures all surface flows from this region and the Temescal Creek watershed. The Santa Ana River headwaters are located in the San Bernardino Mountains in the northeastern part of the watershed. Major tributaries to the Santa Ana River in this region include Warm Creek, Lytle Creek, and San Timoteo Creek. In the north central portion several major Santa Ana River tributaries arise in the San Gabriel Mountains and drain generally south into the Chino Basin before their confluence with the Santa Ana River, including Day Creek, Cucamonga Creek and San Antonio Creek. Many of these drainages carry little to no flow during dry conditions because of the presence of extensive recharge basins in this region. Prado Basin above Prado Dam is a flood control basin that captures all flows from the upper part of the Santa Ana River Watershed. For the most part the basin is an undisturbed, dense riparian wetland.

- *Santa Ana River below Prado Dam and Coastal Plains Region* – This area covers the western portion of the Santa Ana River watershed and includes coastal waterbodies that are not part of the Santa Ana River drainage area. This area is located within Orange County. Below Prado Dam the Santa Ana River flows through the Santa Ana Mountains before crossing the coastal plain and emptying into the Pacific Ocean near Huntington Beach. Groundwater recharge areas near the City of Anaheim capture water in the Santa Ana River and the Santa Ana River is often dry below this area. Other watersheds on the Coastal Plain include Newport Bay, Anaheim Bay-Huntington Harbour and Coyote Creek.

5.3 Purpose of the QAPP

This QAPP supports the SAR Bacteria Monitoring Plan which was prepared to fulfill three objectives:

- (a) Fulfill the monitoring and surveillance requirements for the 2012 adopted BPA to *Revise Recreation Standards for Inland Freshwaters in the Santa Ana Region*;
- (b) Conduct sampling to support implementation of the MSAR Bacteria TMDL, including requirements to implement a watershed-wide compliance monitoring program and source evaluation programs for urban and agricultural dischargers; and
- (c) Support any additional bacterial indicator monitoring that may be conducted in the watershed to support regional regulatory activities.

6. Project/Task Descriptions

6.1 Work Statement and Produced Products

The following Regional and TMDL Bacteria Monitoring Programs are addressed by this QAPP:

- Regional Monitoring Program
 - Priority 1 - REC1 Tier A Waters
 - Priority 2 – Waterbodies with an Adopted TMDL
 - Priority 3 - 303(d) Listed Waterbodies without an Adopted TMDL
 - Priority 4 – REC2 Only Waterbodies
- TMDL Monitoring Programs
 - MSAR Bacteria TMDL Wet Weather Event Monitoring
 - Urban Source Evaluation Monitoring Program
 - Agricultural Source Evaluation Monitoring Program (AgSEMP)

Following is a description of the monitoring activities associated with each program.

6.2 Regional Monitoring Program

6.2.1 Priority 1 REC1 Tier A Waters

6.2.1.1 Introduction

The purpose of Priority 1 REC1 Tier A waters monitoring is to assess compliance with REC1 use water quality objectives for *E. coli*. For the most part, Priority 1 waters are generally those waterbodies listed in Table 5-REC1-Tiers of the BPA with a Tier A designation and no “N” characterization¹⁸. The potential for human health impacts as a result of exposure to pathogens is highest in REC1 Tier A waters where water contact recreational activities are most likely to occur.

6.2.1.2 Monitoring Sites

Table 6-1 identifies seven waterbodies as REC1 Tier A waters for Priority 1 monitoring (Table 6-1). These waterbodies include four lakes: Big Bear Lake, Lake Perris, Canyon Lake, and Lake Elsinore, and three flowing waters, Santa Ana River Reach 3 (two sites), Lytle Creek (North Fork) and Mill Creek Reach 2. Eight sample sites were selected to assess water quality on these waterbodies, with one site per waterbody except for Santa Ana River Reach 3 where two stations were selected. Five sites are located in Riverside County and three sites are located in San Bernardino County (Figure 6-1).

The two Priority 1 Santa Ana River sites (MWD Crossing and @ Pedley Avenue) are also MSAR Bacteria TMDL compliance sites (Table 6-1). Data collected from these sites will also be used for evaluating compliance with the MSAR Bacteria TMDL.

¹⁸ An “N” designation means “Natural Conditions” and per the BPA, “includes freshwater lakes and streams located in largely undeveloped areas where ambient water quality is expected to be better than necessary to protect primary contact recreational activities regardless of whether such activities actually occur in these waterbodies” (Page 56 in Attachment 2 to the Santa Ana Water Board Resolution R8-2012-0001, as corrected).

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Table 6-1. Priority 1 REC1 Tier A monitoring sites

Site ID	Site Description	RMP Priority	Latitude	Longitude
P1-1	Canyon Lake at Holiday Harbor	1	33.6808	-117.2724
P1-2	Lake Elsinore	1	33.3937	-117.3345
P1-3	Lake Perris	1	33.8614	-117.1908
P1-4	Big Bear Lake at Swim Beach	1	34.2482	-116.9034
P1-5	Mill Creek Reach 2	1	34.0891	-116.9247
P1-6	Lytle Creek (North Fork)	1	34.2632	-117.5053
WW-S1	Santa Ana River Reach 3 at MWD Crossing	1	33.9681	-117.4479
WW-S4	Santa Ana River Reach 3 at Pedley Avenue	1	33.9552	-117.5327

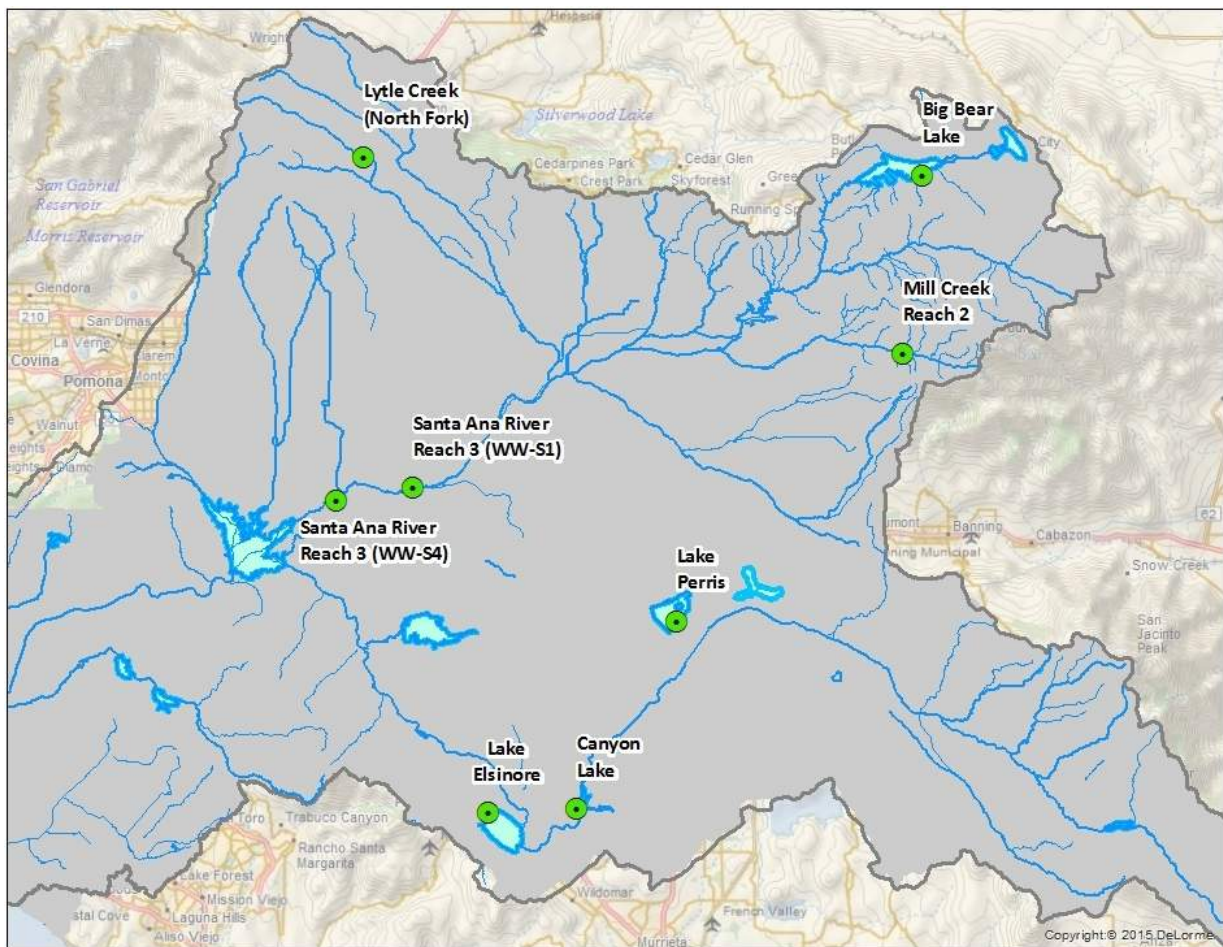


Figure 6-1. Priority 1 REC1 Tier A monitoring sites

6.2.1.3 Sample Frequency

Priority 1 sample sites will be sampled during dry weather (defined as no measurable rainfall within a 72 hour period prior to sampling) for a 20-week period during the warmest part of the year between May 1 and September 30. In addition, Priority 1 sample sites will also be sampled during one 5-week period from end of October through most of November each year during the cooler season. The resulting dataset will include 25 samples each year from each site and provide sufficient data to calculate 16 geometric means during the 20-week sample period and one geometric mean during the cool season. Data will be used to evaluate compliance with:

- Santa Ana region *E. coli* water quality objective: 5-sample/30-day geometric mean of < 126 *E. coli* organisms per 100 mL.
- MSAR Bacteria TMDL dry weather numeric targets for *E. coli*: 5-sample/30-day geometric mean < 113 organisms/100 mL and not more than 10 percent of the samples exceed 235 organisms/100 mL for any 30-day period. The MSAR Bacteria TMDL requires compliance with the dry weather numeric targets by December 31, 2015.

While it is unlikely that ice conditions will occur during each year's cool season sample period, if ice conditions prevent sampling at a Priority 1 site, that finding will be documented on the field form and photo documentation will be provided.

6.2.2 Priority 2 – Waterbodies with an Adopted TMDL

6.2.2.1 Introduction

The purpose for monitoring Priority 2 waters is to evaluate attainment of water quality objectives in waters that have an adopted bacteria TMDL. Currently, only one bacteria TMDL has been adopted for inland waters in the watershed: MSAR Bacteria TMDL. Dry weather sampling has been ongoing in these waters since 2007 to satisfy TMDL implementation requirements. This dry weather sampling will continue as described in this section of the RMP; any other monitoring necessary to satisfy TMDL requirements, e.g., wet weather event sampling is described in Section 6.3.1.

6.2.2.2 Monitoring Sites

Monitoring for Priority 2 waters will occur at the same five monitoring sites previously established for evaluating compliance with the numeric targets in the MSAR Bacteria TMDL: Two Santa Ana River Reach 3 sites (@ MWD Crossing and @ Pedley Avenue), and one site each on Mill-Cucamonga Creek, Chino Creek, and Prado Park Lake¹⁹ (Table 6-2; Figure 6-2). As discussed in Section 6.2.1.2, the two Santa Ana River sites are also Priority 1 waters, i.e., as Tier A waters they are locations where the risk of exposure to pathogens during recreational activities is highest. Both Figure 6-2 and Table 6-2 indicate the dual designation for these sites. With the exception of the Mill-Cucamonga Creek monitoring site, the location of each sample site remains the same as previously sampled under the MSAR Bacteria TMDL. The Mill-Cucamonga Creek site has been moved to take into account changes in the local area, resulting from the completion of the Mill Creek Wetlands.

¹⁹ See Monitoring Plan Section 4.1.1 for the original basis for the selection of these monitoring sites.

Table 6-2. Priority 2 monitoring sites (Note that WW-S1 and WW-S4 sites are also Priority 1 sites)

Site ID	Site Description	RMP Priority	Latitude	Longitude
WW-M6	Mill-Cucamonga Creek below Wetlands	2	33.9268	-117.6250
WW-C7	Chino Creek at Central Avenue	2	33.9737	-117.6889
WW-C3	Prado Park Lake	2	33.9400	-117.6473
WW-S1	Santa Ana River Reach 3 at MWD Crossing	1,2	33.9681	-117.4479
WW-S4	Santa Ana River Reach 3 at Pedley Avenue	1,2	33.9552	-117.5327

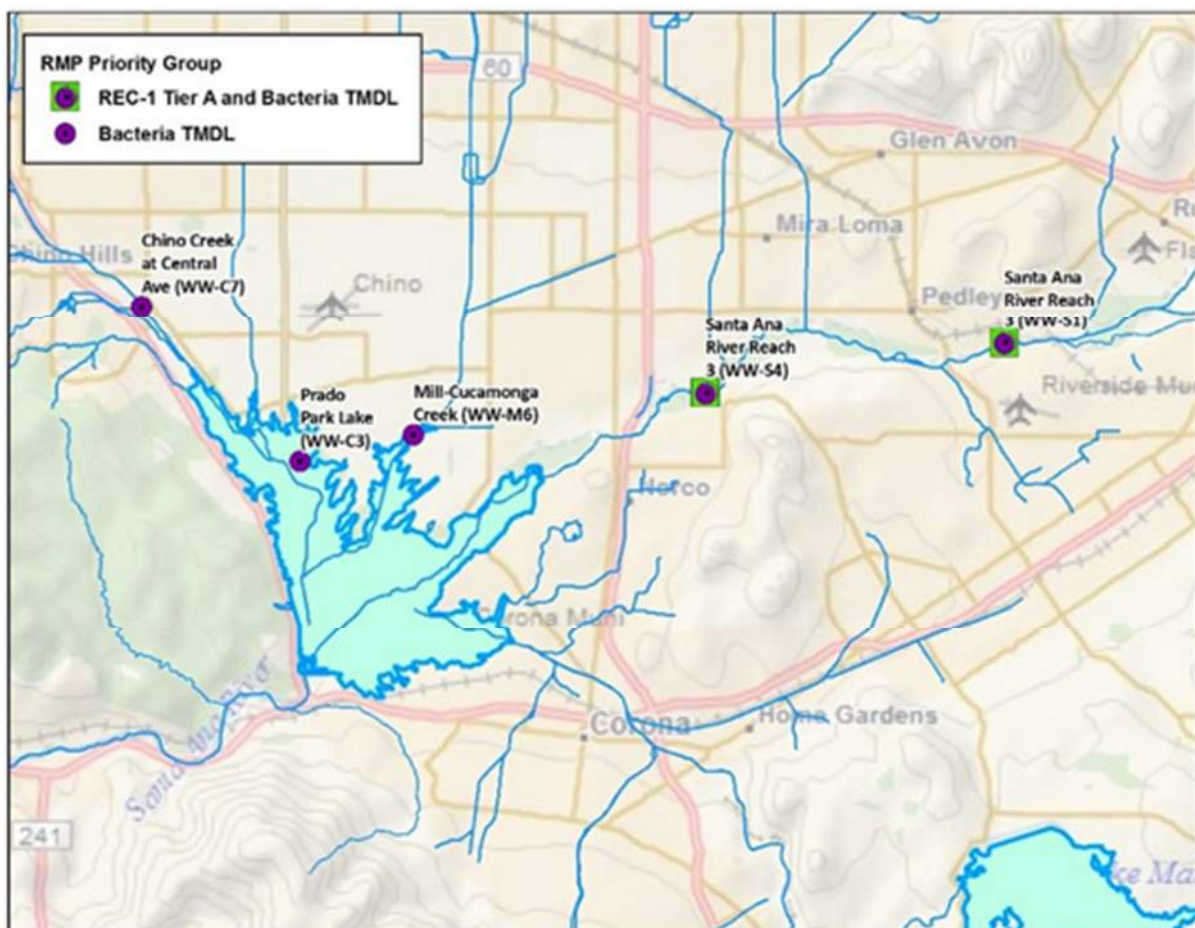


Figure 6-2. Priority 2 monitoring sites (note that the two monitoring sites on the Santa Ana River are also Priority 1 sites, see text for explanation)

6.2.2.3 Sample Frequency

The sampling frequency for dry weather (defined as no measurable rainfall within a 72 hour period prior to sampling) for Priority 2 waters is the same as described for Priority 1 waters in Section 6.2.1.3. Any additional monitoring required to satisfy MSAR Bacteria TMDL-specific requirements, e.g., wet weather event monitoring, is described in Section 6.3.1 below.

6.2.3 Priority 3 – 303(d) Listed Waterbodies without Adopted TMDL

6.2.3.1 Introduction

Priority 3 waters are those that have been listed as impaired for bacterial indicators and have been placed on the state's 303(d) List, but do not have an adopted TMDL. The most recent EPA-approved list of impaired waters is based on the State Water Board's 2010 Integrated Report²⁰. These waters can be removed from the 303(d) List (per the requirements of the State Water Board's Listing Policy), if water quality data indicate that removal from the list is appropriate; otherwise, it is anticipated that a TMDL will be established for Priority 3 waters in the future. The purpose for monitoring these waters is gather data to support eventual regulatory decisions regarding the degree of impairment in each Priority 3 waterbody (e.g., to support a delisting decision).

6.2.3.2 Monitoring Sites

In the Santa Ana River watershed 21 waterbodies are currently on the 303(d) List with no adopted TMDL: twelve in Orange County; four in Riverside County, and five in San Bernardino County (see Table 1.1 in the Monitoring Plan). The following waterbodies have not been included in this RMP as Priority 3 waterbodies for the following reasons:

- Silverado Creek in Orange County is expected to be delisted in the next 303(d) listing cycle;
- Santa Ana Delhi Channel in Orange County because REC1 has been removed as a beneficial use by an approved use attainability analysis and the 303(d) listing is based on REC1; accordingly this waterbody considered a Priority 4 waterbody under this RMP;
- Canyon Lake in Riverside County is also classified as a Priority 1 waterbody; in addition, work carried out by others has shown that bacterial indicator concerns in this waterbody are associated with wet weather conditions;
- Temescal Creek Reach 6 in Riverside County is a listing error.
- The 303(d) listing for Knickerbocker Creek in San Bernardino County is being addressed through that county's MS4 Permit (R8-2010-0036); recent studies have shown that impairment is due to wildlife concentration.
- Lytle Creek and Mill Creek Reach 2 in San Bernardino County are also designated as a Priority 1 waterbody in this RMP.
- Mill Creek Reach 1 is an old listing and there is no data available that provides the original basis for its current listing as impaired. In addition, this reach is designated with an intermittent REC1 beneficial use and a recent reconnaissance found no surface water. Given the likelihood that REC1

²⁰ The final list which includes waterbodies added to the list by EPA Region 9 is found here:
http://www.waterboards.ca.gov/water_issues/programs/tmdl/2010state_ir_reports/category5_report.shtml

activity would be limited in this reach and more likely to occur in the upstream Reach 2, this waterbody was not included as a Priority 3 waterbody.

Figure 6-3 shows the general location for each of Priority 3 waterbody in each county. Selection of a sample site for each waterbody relied on the following criteria:

- One sample site per waterbody, unless there is a compelling need for a second site, e.g., significant differences exist in the waterbody's characteristics in different reaches;
- Site should be close to areas of existing or potential water contact recreational activities;
- For sites near the Pacific Ocean, site is upstream of the tidal prism; and
- If possible, maintain historical monitoring sites.

Table 6-3 provides a brief description of each site, including known water quality data and basis for 303(d) listing, and Attachment A, Section A.3, of the SAR Bacteria Monitoring Plan provides detailed descriptions and photographs for each site.

6.2.3.3 Sample Frequency

Water quality samples will be collected during dry weather (defined as no measurable rainfall within a 72 hour period prior to sampling) according to the frequency shown in Table 10-2. The overall sample schedule for these sites overlaps with the Priority 1 & 2 sample site schedule to maximize efficiency with the collection of samples. The resulting dataset for these sites will consist of five samples per year from each site. Data from each year will represent a different five week period.

6.2.4 Priority 4 – REC2 Only Waterbodies

6.2.4.1 Introduction

Priority 4 waters are those where the REC1 beneficial use has been removed as a result of an approved use attainability analysis (UAA). The applicable *E. coli* or *Enterococcus* water quality objectives for these waters are based on antidegradation targets established by the BPA²¹. Currently, there are four inland freshwaters with a REC2 only designation: Temescal Creek (Reaches 1a and 1b; Riverside County); Santa Ana Delhi Channel (Tidal Prism and Reaches 1 and 2; Orange County); Greenville-Banning Channel (Tidal Prism Reach, Orange County); and Cucamonga Creek (Reach 1, San Bernardino County).

²¹ The BPA presents antidegradation targets and describes the statistical methodology employed to develop the numeric values. In short, historical data was fitted to a lognormal distribution, and the 75th percentile of the fitted lognormal distribution was selected as the antidegradation target. Accordingly, the 75th percentile of the fitted log-normal distribution for a newly acquired dataset with comparable spatial (within reach) and temporal (seasonal) variability, should be less than or equal to that of the historical dataset.

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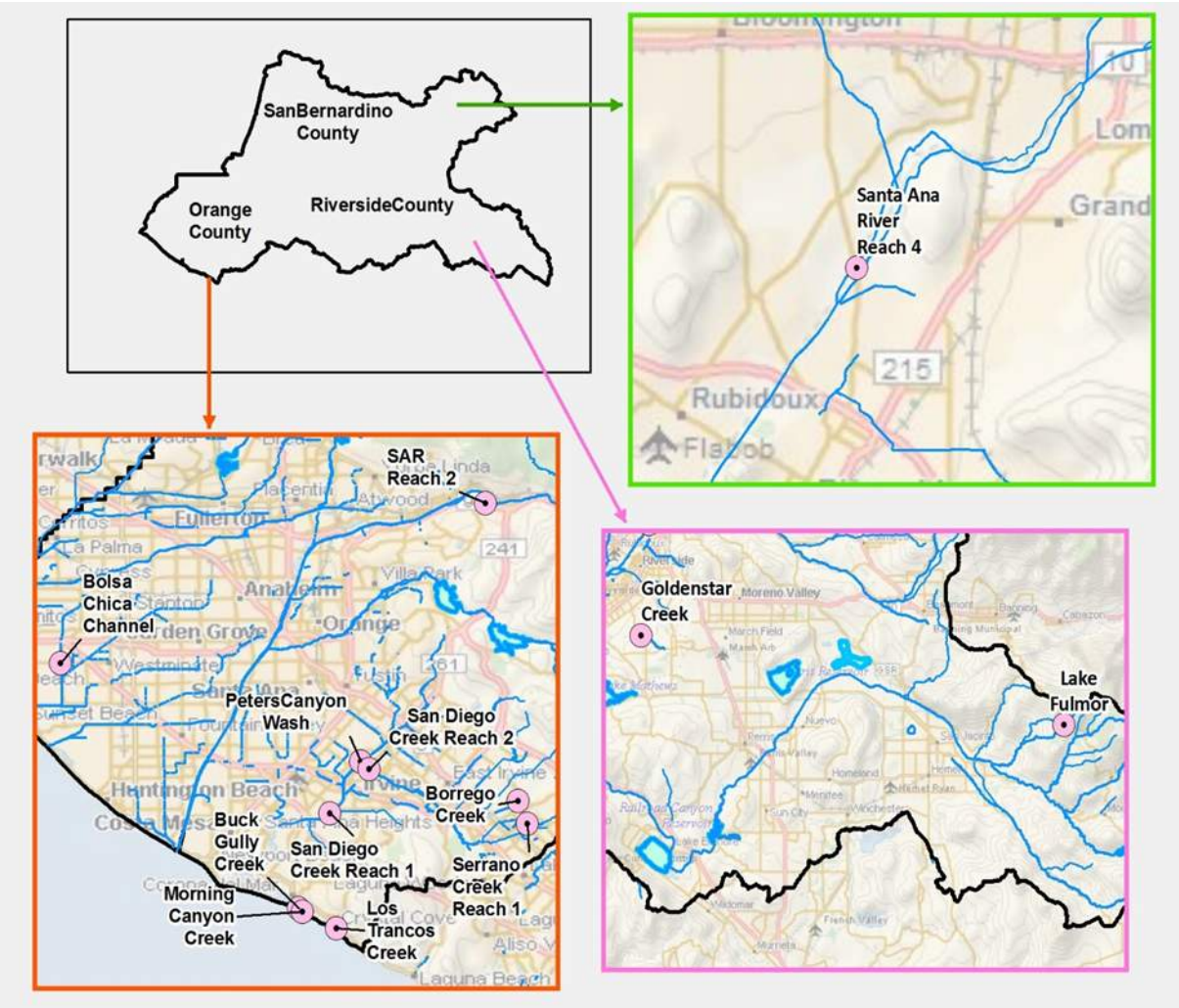


Figure 6-3. Priority 3 monitoring sites by County within the Santa Ana River watershed.

Table 6-3. Priority 3 monitoring sites and the basis for 303(d) listing.

Site ID	Site Description	Latitude	Longitude	Frequency of <i>E. coli</i> Exceedance ^{1,2}	Comments ²
P3-OC1	Bolsa Chica Channel upstream of Westminster Blvd/Bolsa Chica Rd	33.75958	-118.04295	<i>E.coli</i> 49/63	Based on Orange County Coastkeeper Coastal Watersheds Project Report. Data collected between March 31, 2004 and March 30, 2006 from two sites: "bc1" – in Cypress in the upper Bolsa Chica Channel at Warland Street Bridge; "bc2" – in Huntington Beach in the lower Bolsa Chica Channel at the intersection of Bolsa Chica Rd. and Rancho Rd.
P3-OC2	Borrego Creek upstream of Barranca Parkway	33.65457	-117.73213	<i>E.coli</i> 37/43	Based on Orange County Coastkeeper Coastal Watersheds Project Report. Data collected between March 11, 2004 and March 29, 2006 from two sites: "bor1" – in Foothill Ranch in upper Borrego Channel on Town Center Dr.; "bor2" – in Irvine in the lower Borrego Channel on Barranca Pkwy next to the train station.
P3-OC3	Buck Gully Creek Little Corona Beach at Poppy Avenue/Ocean Blvd	33.59000	-117.86841	<i>E.coli</i> 23/68	303(d) list states that listing is for reach downstream of Pacific Coast Highway; state website states that listing decision made prior to 2006 and there is no information in state assessment database. However, Orange County Coastkeeper (OCC) database shows two sites labeled "bg1" and "bg2" that were sampled from March 8, 2004 to April 13, 2006 (exceedance frequency in this table based on those results); no information in OCC database regarding where sites are located.
P3-OC5	Los Trancos Creek at Crystal Cove State Park	33.57601	-117.84062	Fecal coliform 5/9	303(d) list states that listing is for reach downstream of Pacific Coast Highway; state website states that listing decision made prior to 2006 and there is no information in state assessment database. However, data obtained from Regional Board shows three sample locations sampled for fecal coliform in July and September in 2000. All exceedances (5 of 9) occurred at a sample site adjacent to the most upstream golf cart bridge of the Pelican Hill Golf Course
P3-OC6	Morning Canyon Creek at Morning Canyon Beach	33.58759	-117.86575	<i>E.coli</i> 17/61	Based on Orange County Coastkeeper Coastal Watersheds Project. Data collected between March 8, 2004 and April 10, 2006 from two sites: "mc1" – in Newport Beach in the upper part of Morning Canyon Creek at Surrey street; "mc2" – in Newport Beach in the lower part of Morning Canyon Creek at Morning Canyon Beach.
P3-OC7	Peters Canyon Wash downstream of Barranca Parkway	33.69076	-117.82404	<i>E.coli</i> 40/66	Based on Orange County Coastkeeper Coastal Watersheds Project. Data collected between March 9, 2004 and March 29, 2006 from two sites: "pc1" – in Irvine in upper Peter's Canyon Channel on Bryan Street between Jamboree Rd. and Culver Dr.; "pc2" – in Irvine in lower Peter's Canyon Channel on Barranca Pkwy between Jamboree Rd. and Harvard Ave.
P3-OC8	San Diego Creek downstream of Campus Drive (Reach 1)	33.65530	-117.84535	<i>E.coli</i> 33/84	State website states that listing decision made prior to 2006 and there is no information in state assessment database. However, based on Orange County Coastkeeper Coastal Watersheds Project, data was collected between October 22, 2002 and June 21, 2004 from three sites: "sd4", "sd5", and "sd6". Exceedance frequency shown in this table is from OCC report; no information available on specific sample locations.

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Table 6-3. Priority 3 monitoring sites and the basis for 303(d) listing.

Site ID	Site Description	Latitude	Longitude	Frequency of <i>E. coli</i> Exceedance ^{1,2}	Comments ²
P3-OC9	San Diego Creek at Harvard Avenue (Reach 2)	33.689772	117.8186854	<i>E.coli</i> 31/64	Based on Orange County Coastkeeper Coastal Watersheds Project. Data collected between October 22, 2002 and June 21, 2004 from three sites: “sd1” – Bake Parkway, site is located off of Irvine Center Dr. on the right hand side before Wild Rivers water park; “sd2” – 133 Fwy, the 133 Fwy is located off of Pacifica and Alton in the dead end down the ramp in the riverbed; and “sd3”- Sand Canyon, site is located off of the 405 Fwy at Sand Canyon Avenue past Alton at the bridge on the NE corner of Barranca and Sand Canyon Avenue.
P3-OC10	Santa Ana River Reach 2 downstream of Imperial Highway	33.857440	-117.791617	<i>E.coli</i> 37/150	Based on Orange County Coastkeeper Coastal Watersheds Project. Data collected between October 28, 2002 and June 10, 2004 from six sites: “sar1” – Green River, off the 91 Fwy at Green River exit under the bridge at the golf course; “sar2” – Gypsum Canyon, off of Gypsum Canyon Rd. and Yorba Linda Blvd, across street from the “Fantasy Restaurant”; “sar3” – Yorba Linda Park, Santa Ana River east of Lakeview St. off of the 91 Fwy; “sar4” – Lakeview, off the 91 Fwy and Lakeview St. across from Kaiser Permanente; “sar5” – Lincoln, off 57 Fwy N before it meets the 91 Fwy off of Lincoln St. on the NE corner of the bridge; and “sar6” – Katella, off the 57 Fwy N at the Katella exit, across from the Anaheim Pond Sports arena.
P3-OC11	Serrano Creek upstream of Barranca/Alton Parkway	33.64870	-117.72442	<i>E.coli</i> 35/68	Based on Orange County Coastkeeper Coastal Watersheds Project. Data collected between March 11, 2004 and March 29, 2006 from two sites: “ser1” – in Forest Grove in the upper Serrano Channel in Trabuco Rd and Peachwood under the bridge; “ser2” – in Irvine in the lower Serrano Channel, next to the Alton/Barranca intersection.
P3-RC1	Goldenstar Creek at Ridge Canyon Drive	33.8964	-117.3586	<i>E.coli</i> 19/79	Based on Orange County Coastkeeper Coastal Watersheds Project. Data collected between October 29, 2002 and June 3, 2004 from three sites: “gs1” – near the intersection of Van Buren Boulevard and Wood Road in City of Riverside; “gs2” – located at the end of Ridge Run Road in City of Riverside; and “gs3” – downstream of Golden Star Creek Road in City of Riverside. Exceedances at gs1 and gs2 only.
P3-RC2	Lake Fulmor at the Lakeside Boardwalk	33.8052	-116.7798	Data unavailable	State website states that listing decision made prior to 2006 and there is no information in state assessment database.
P3-SBC1	Santa Ana River Reach 4 above S. Riverside Avenue Bridge	34.0248	117.3628	Data unavailable	State website states that listing decision made prior to 2006 and there is no information in state assessment database.

¹ X/Y = First number is the number of exceedances; the second number is the number of samples.

² Source for information regarding exceedances is (a) the State Water Board’s website for 2010 Integrated Report:

http://www.waterboards.ca.gov/water_issues/programs/tmdl/2010state_ir_reports/category5_report.shtml (find the relevant waterbody and click on the specific pollutant for summary of available data and listing history.; and (b) Santa Ana River Citizen Monitoring Project Final Report (“Orange County Coastkeeper Coastal Watersheds Project”, November 2004.

6.2.4.2 Monitoring Sites

The monitoring sites for each Priority 4 waterbody are as follows (see Table 6-4, Figure 6-4 and SAR Bacteria Monitoring Plan, Section A.4 in Attachment A for additional location information):

- *Santa Ana Delhi Channel* – The Santa Ana Delhi Channel has two reaches that are REC2 only: (a) Reach 2 is within the City of Santa Ana, Orange County, CA and extends from Sunflower Avenue upstream to Warner Avenue, a distance of approximately 1 mile; (b) Reach 1 is within the cities of Costa Mesa and Newport Beach, CA and extends from the tidal prism upstream to Sunflower Avenue, a distance of approximately 2.5 miles. Two monitoring sites have been selected for the Santa Ana Delhi Channel to provide sample results from freshwater and tidal prism areas: (a) Upstream of Irvine Avenue; and (b) within the tidal prism at the Bicycle Bridge.
- *Greenville-Banning Channel Tidal Prism Segment*– This segment of the Greenville-Banning channel is designated REC2 only. It begins at its confluence with the Santa Ana River and extends upstream approximately 1.2 mile to the inflatable rubber dam operated by the Orange County Public Works Department. The monitoring site is located at an access ramp approximately 60 meters downstream of the trash boom below the rubber diversion dam.
- *Temescal Creek* – Temescal Creek has two reaches that are REC2 only: (a) Reach 1a is within the City of Corona, Riverside County and extends from Lincoln Avenue to confluence with Arlington Channel, a distance of approximately 3 miles; (b) Reach 1b within City of Corona and extends from Arlington Channel confluence to 1400 feet (ft) upstream of Magnolia Avenue (City of Corona). The monitoring site for Temescal Creek is located upstream of the Main Street Bridge.
- *Cucamonga Creek Reach 1* – Cucamonga Creek Reach 1 extends from the confluence with Mill Creek in the Prado area to near 23rd Street in the City of Upland. The monitoring site for Cucamonga Creek Reach 1 is at Hellman Road.

6.2.4.3 Sample Frequency

Water quality samples will be collected during dry weather (defined as no measurable rainfall within a 72 hour period prior to sampling) once per year until an *E. coli* or *Enterococcus* result exceeds the antidegradation target threshold value for the site (equal to the 75th percentile of the lognormal distribution fitted to historical data). If an exceedance of the antidegradation target is observed, additional bacterial indicator samples will be collected once/month for the three following months. If any of the follow-up samples exceed the antidegradation target, then sampling will continue on a monthly basis until source(s) of the increased bacterial indicator concentration is identified and mitigated and *E. coli* or *Enterococcus* levels return to below the antidegradation target in three of four samples collected over three consecutive months.

Table 6-4. Priority 4 monitoring sites

Site ID	Site Description	RMP Priority	Latitude	Longitude
P4-RC1	Temescal Creek at Main Street	4	33.8895	-117.5636
P4-OC1	Santa Ana Delhi Channel Upstream of Irvine Avenue	4	33.6602	-117.8810
P4-OC2	Santa Ana Delhi Channel in Tidal Prism	4	33.6529	-117.8837
P4-OC3	Greenville-Banning Channel in Tidal Prism	4	33.6594	-117.9479
P4-SBC1	Cucamonga Creek at Hellman Avenue	4	33.9493	-117.6104

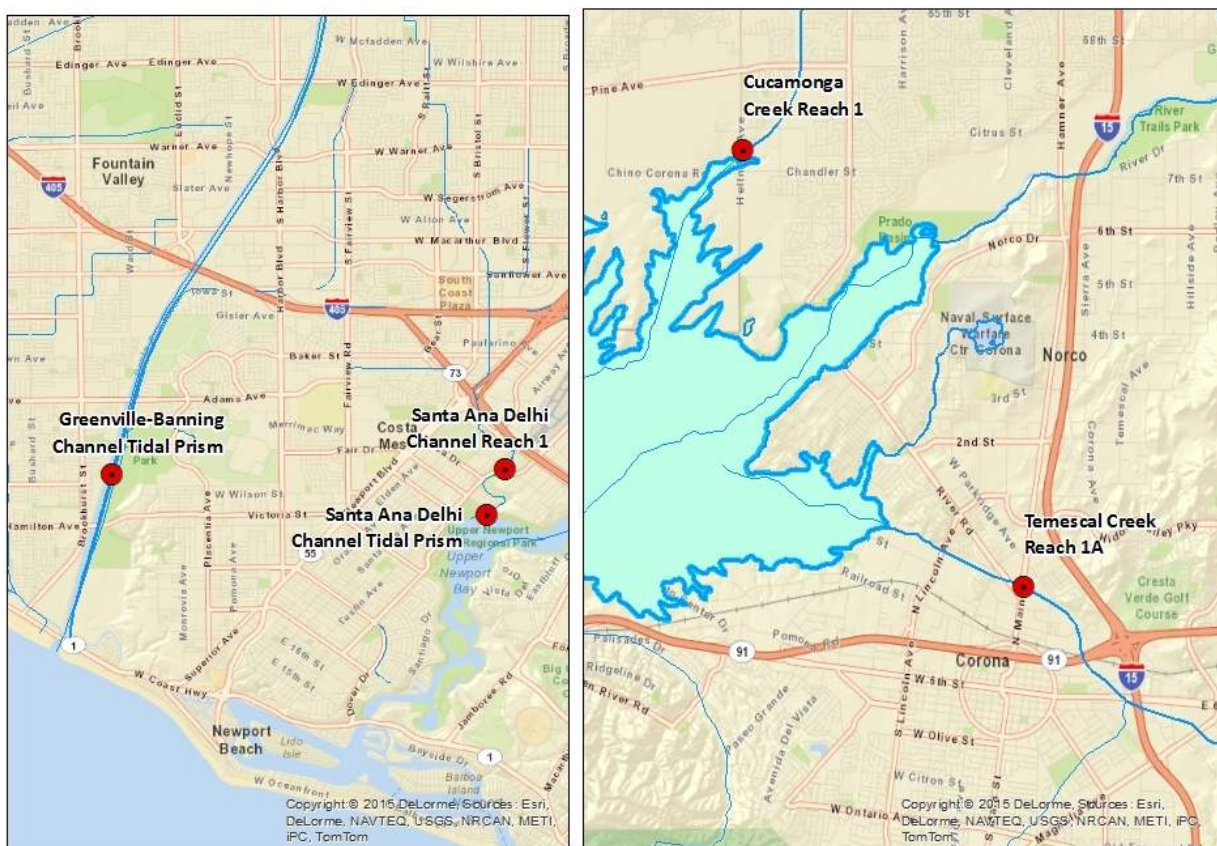


Figure 6-4. Priority 4 monitoring sites by County within the Santa Ana River watershed.

6.3 TMDL Monitoring Program

6.3.1 MSAR Bacteria TMDL Wet Weather Event Monitoring

6.3.1.1 Introduction

The purpose of the MSAR Bacteria TMDL watershed-wide compliance monitoring program is to assess compliance with wasteload allocations in the MSAR Bacteria TMDL (see Section 5.1.2). Compliance monitoring for the MSAR Bacteria TMDL during dry weather is addressed by monitoring conducted for Priority 1 and Priority 2 (described above in Sections 6.2.1 and 6.2.2, respectively). The MSAR Bacteria TMDL also requires the collection of bacteria water quality samples during one wet weather

event each year. Monitoring for one storm event per wet season is carried out as a component of the TMDL Monitoring Program.

The same concentration based TMDL wasteload and load allocations for *E. coli* apply to both wet weather and dry weather conditions:

- *MSAR Bacteria TMDL wet weather numeric targets for E. coli*: 5-sample/30-day geometric mean < 113 organisms/100 mL and not more than 10 percent of the samples exceed 235 organisms/100 mL for any 30-day period.

Per the MSAR Bacteria TMDL, compliance with these numeric targets during wet weather shall be achieved by December 31, 2025. The TMDL allowed for an extended compliance timeline for wet weather conditions, because of the “expected increased difficulty in achieving compliance under [wet weather] conditions”²².

6.3.1.2 Monitoring Sites

Table 6-5 and Figure 6-5 identify the monitoring sites for evaluating compliance with MSAR Bacteria TMDL numeric targets during wet weather.

6.3.1.3 Sample Approach and Frequency

One wet weather event is targeted for sampling each wet season, defined as November 1 through March 31 in the MSAR Bacteria TMDL. The goal of wet weather event sampling is to collect bacterial indicator data during the rising and falling limbs of the hydrograph. To accomplish this goal, a wet weather sample event requires the collection of four samples over an approximately four day period:

- *Sample 1* – Target sample collection on the day of the storm event when it is apparent that flow within the channel is elevated above typical dry weather conditions as a result of rainfall induced runoff.
- *Sample 2* – Collect samples approximately 48 hours after collection of Sample 1.
- *Sample 3* – Collect samples approximately 72 hours after collection of Sample 1.
- *Sample 4* – Collect samples approximately 96 hours after collection of Sample 1.

The decision whether to conduct wet weather sampling will be made by implementing the following steps:

- *Step 1* - Prepare to deploy the sampling team if rain is forecast (National Weather Service forecast on <http://www.Accuweather.com>), i.e., the sample teams are put on stand-by;
- *Step 2* - If rain develops, monitor rain gauges in the area (Riverside Municipal Airport and Ontario International Airport); and

²² Page 3 of 15, Attachment A to Santa Ana Water Board Resolution R8-2005-0001

Table 6-5. MSAR Bacteria TMDL wet weather event monitoring sites

Site ID	Site Description	Latitude	Longitude
WW-M6	Mill-Cucamonga Creek below Wetlands	33.9410	-117.6209
WW-C7	Chino Creek at Central Avenue	33.9737	-117.6889
WW-C3	Prado Park Lake	33.9400	-117.6473
WW-S1	Santa Ana River Reach 3 at MWD Crossing	33.9681	-117.4479
WW-S4	Santa Ana River Reach 3 at Pedley Avenue	33.9552	-117.5327

Coordinates are shown as Geographic WGS 1984 World Datum

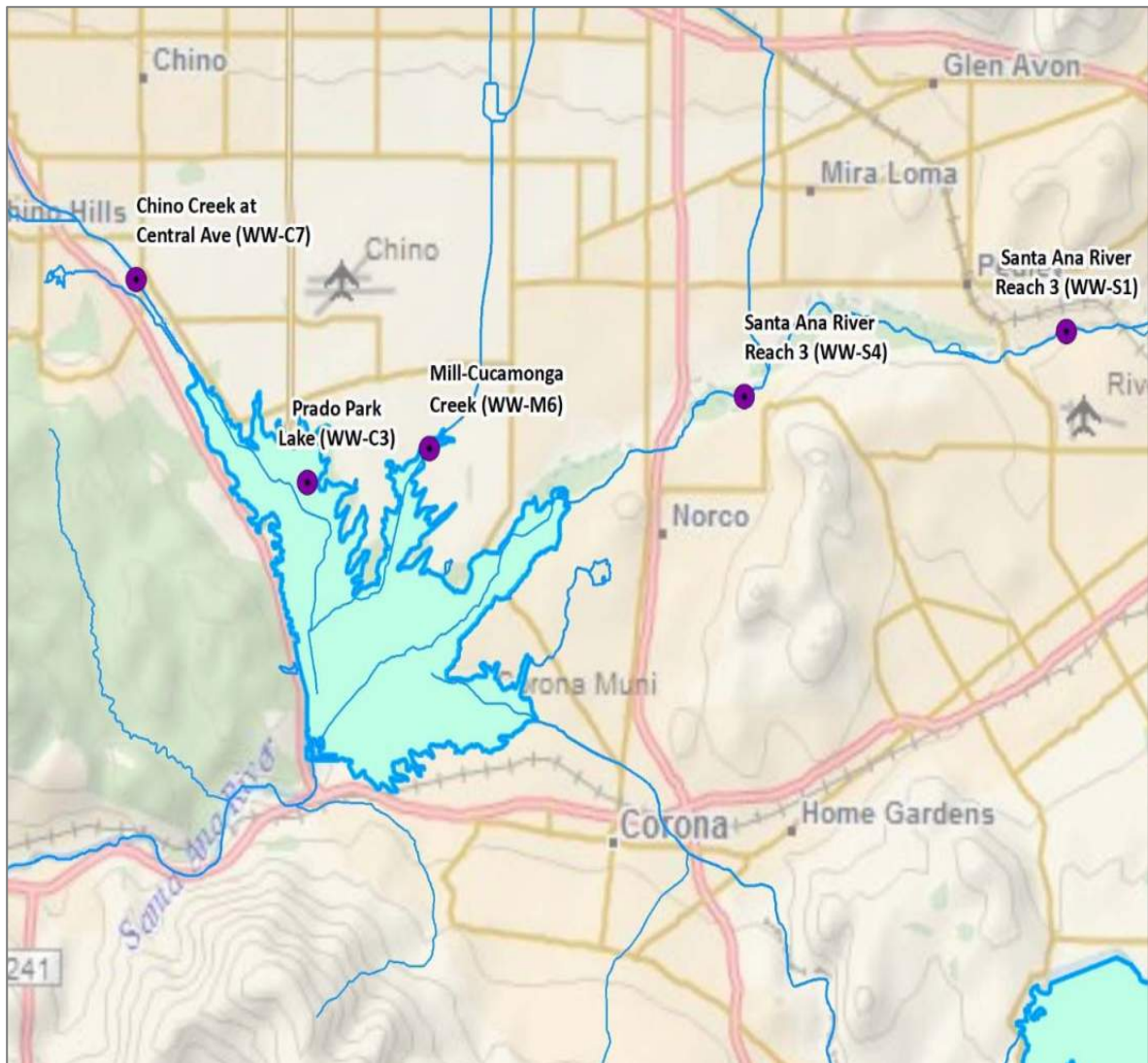


Figure 6-5. MSAR Bacteria TMDL wet weather event monitoring sites

- *Step 3* - Mobilize sampling crews at first daylight on the appropriate morning for sampling based upon the time that rainfall is expected. For instance, if rainfall onset is predicted for 0400 hours, samplers will be mobilized so that they arrive at sampling sites by daylight on the day of the predicted rainfall. If rainfall is predicted for 1300 hours, then samplers will mobilize at daylight of the next morning. Regardless of when rainfall begins, mobilization of sample teams is limited to first daylight to meet two sampling requirements:
 - For safety purposes, sampling may only be conducted during daylight hours; and
 - Samples must be dropped off at the laboratory, typically no later than 1500 hours to comply with laboratory processing procedures and to meet holding times.

Samples shall not be collected if conditions are determined to be unsafe by an on-site assessment conducted by the field team leader. If a wet weather event occurs during weekends or holidays, then additional coordination with the laboratory will be necessary to ensure water samples can be accepted for processing.

6.3.2 Urban Source Evaluation Program

6.3.2.1 Introduction

The MSAR Bacteria TMDL required MS4 dischargers to develop a USEP by November 30, 2007, six months after EPA approval of the MSAR TMDL. The purpose of the USEP was to identify specific activities, operations, and processes in urban areas that contribute bacterial indicators to waterbodies under the MSAR Bacteria TMDL. Prepared through the MSAR TMDL Task Force, the USEP was submitted to the Santa Ana Water Board in a timely manner and formally approved on April 18, 2008²³. The approved USEP included the following objectives:

- Describe an Urban Source Evaluation Monitoring Program to be implemented to identify urban bacterial indicator sources;
- Establish a risk-based framework for evaluating water quality data obtained with regards to human illness from the Urban Source Evaluation Monitoring Program;
- Identify investigative activities that may be implemented to the maximum extent practicable based on water quality data; and
- Provide a schedule for USEP implementation with contingencies built in to allow for consideration of new data, modified regulations, changed priorities, or new technologies.

On January 29, 2010 the Santa Ana Water Board adopted new MS4 permits for Riverside and San Bernardino Counties. These permits required that each County develop a CBRP to meet MSAR TMDL wasteload allocations for the dry season. The source evaluation activities described in the USEP were incorporated into the CBRP. Accordingly, following Santa Ana Water Board approval of the CBRPs for each County on February 10, 2012²⁴, the CBRPs superseded the previously approved USEP and became the basis for bacterial indicator urban source evaluation activities carried out in the MSAR

²³ Santa Ana Water Board Resolution: R8-2008-0044; April 18, 2008

²⁴ Santa Ana Water Board Resolutions: R8-2012-0015 (Riverside County MS4 Program; R8-2012-0016 (San Bernardino County MS4 Program)

watershed (see page A-11 in the Riverside County CBRP; similar language is contained in the San Bernardino County CBRP)²⁵.

The Los Angeles Regional Water Quality Control Board (Los Angeles Water Board) adopted a new Los Angeles County MS4 Permit in 2012 that became effective December 28, 2012²⁶. This permit required the Cities of Pomona and Claremont to develop CBRPs for the portions of their cities that are within the MSAR watershed²⁷. Because the Santa Ana Water Board oversees MSAR Bacteria TMDL implementation, the Santa Ana Water Board oversaw development of the CBRPs for these cities. The Santa Ana Board approved the CBRPs for the Cities of Pomona and Claremont on March 14, 2014²⁸.

The primary goal of the source evaluation monitoring program is to guide efforts to identify and where possible mitigate controllable sources of bacterial indicator derived from discharges covered by MS4 permits. Source evaluation activities seek to answer the following questions:

- Which subwatershed areas are hydrologically connected to the waterbodies listed as impaired (in particular the Santa Ana River) by the MSAR Bacteria TMDL during dry flow conditions?
- What is the concentration of *E. coli* and rate of urban dry weather flow from MS4 facilities outfalls to a downstream TMDL compliance monitoring sites?
- What is the running geometric mean of *E. coli* in water samples collected from MS4 facilities?

The CBRPs establish an implementation approach to address these questions through source evaluation monitoring and elimination of controllable bacteria sources.

6.3.2.2 CBRP Implementation Approach

The MS4 permittees in each county implement source evaluation activities using a comprehensive, methodical approach that provides data to make informed decisions regarding the potential for an MS4 outfall or group of outfalls to discharge controllable sources of bacterial indicators. This approach relies on the following activities:

- *Tier 1 Reconnaissance* – Tier 1 sites are defined as sites where urban sources of dry weather flow may directly discharge to a downstream watershed-wide compliance site (see Table 6-6). Some of the Tier 1 sites are at the same sites sampled as part of implementation of the USEP in 2007-2008. Additional Tier 1 sites were included, where needed, to supplement existing information. Some Tier 1 locations were dry or had minimal dry weather flow, or in some instances were hydrologically disconnected to downstream waters. The data collected during Tier 1 was used to determine each outfall's potential to contribute controllable sources of bacterial indicators.
- *Prioritization of MS4 Drainage Areas* – Based on the findings from Tier 1 reconnaissance activities, MS4 drainage areas with potentially controllable urban sources of bacterial indicators are prioritized based on factors such as the magnitude of bacterial indicator concentrations and

²⁵ CBRPs available at http://www.waterboards.ca.gov/santaana/water_issues/programs/tmdl/msar_tmdl.shtml

²⁶ Los Angeles Water Board Resolution R4-2012-0175

²⁷ See Attachment R, Los Angeles Water Board Resolution R4-2012-0175

²⁸ Santa Ana Water Board Resolution: R8-2014-0030 (City of Claremont); R8-2014-0031 (City of Pomona)

results from source tracking analyses. Areas with controllable sources of bacteria (as determined through the use of *Bacteroides* testing for human marker) receive the highest priority for action.

- **Tier 2 Source Evaluation** – Source evaluation activities are being implemented first in the MS4 drainage areas with the highest priority Tier 1 sites. These activities include a strategically timed mix of field reconnaissance, secondary screening tool deployment, and bacterial water quality sample collection. Tier 2 sites are tributary to a Tier 1 site. Implementation of source evaluation activities at Tier 2 sites can be unique and is tailored for each drainage area. This ensures that source evaluation activities are as effective as possible given the large amount of potential monitoring sites within large urbanized drainage areas to an MS4 outfall. Methods for conducting Tier 2 source evaluation studies are provided in Section 11 of this QAPP.

The frequency of sample collection at any Tier 1 or Tier 2 site is determined by the need for source evaluation data to identify controllable sources of bacterial indicators.

6.3.2.3 Monitoring Sites

Table 6-6 lists the 34 Tier 1 locations that comprise all of the MS4 outfalls with existing or potential dry weather flow (Figure 6-6). These sites were recommended for sampling in the CBRPs prepared for Riverside and San Bernardino Counties, and the Cities of Pomona and Claremont, located in Los Angeles County.

For Tier 2, the selection of sample sites is determined by the characteristics of the drainage area upstream of the prioritized MS4 outfalls. As a consequence, there is no list of specific sites for Tier 2 source evaluations. Based on the Tier 1 reconnaissance in 2011-2012, the subwatersheds to the Tier 1 sites shown in Table 6-7 were the subject of Tier 2 source evaluation in the 2013 and 2014 dry seasons. The schedule contained in the CBRPs all provided for two dry seasons of Tier 2 source evaluation (2013, 2014). Future Tier 2 source evaluation will be conducted as the MS4 Permittees continue the process of tracking down controllable sources of bacterial indicators within MS4s.

6.3.2.4 Sampling Frequency

Within the MS4 drainage areas there is a vast drainage system that would be nearly impossible to completely monitor in a timely basis using water quality sample collection and analysis alone. To optimize resources, alternative monitoring methods have been identified that are recommended for use to track controllable sources of human fecal bacteria in prioritized MS4 drainage areas. Many of these methods are adapted from a Center for Watershed Protection guidance document²⁹.

Two bacteria source evaluation approaches are available for use by any MS4 permittee within any high priority drainage area, referred to as broad-brush or subregional approaches. The difference in these approaches involves the order of different types of investigation and the number of sites and frequency of water quality sample collection. Each approach is described below.

²⁹ Brown, E., Caraco, D., Pitt, R. 2004. *Illicit Discharge Detection and Elimination: Technical Appendices*. Center for Watershed Protection, Ellicott City, MD & University of Alabama, Tuscaloosa, AL.

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Table 6-6. Tier 1 sample sites in the MSAR watershed¹

Site ID	Site Description	Longitude	Latitude
Riverside County			
T1-64ST	64th Street Storm Drain (SAR Reach 3)	-117.488532	33.970798
T1-ANZA	Anza Drain (SAR Reach 3)	-117.463100	33.95869
T1-BXSP	Box Springs Creek @ Tequesquite Ave	-117.403599	33.975899
T1-CREST	City of Riverside Outfall (Crest/Ontario) (SAR Reach 3)	-117.476290	33.963361
T1-IDST	City of Riverside (Industrial/Freemont) (SAR Reach 4)	-117.436110	33.967330
T1-EVAN	City of Riverside Outfall (Lake Evans) (SAR Reach 4)	-117.381757	33.997002
T1-RBDX	City of Riverside Outfall at Rubidoux (SAR Reach 3)	-117.410220	33.968060
T1-DAY	Day Creek	-117.532980	33.975010
T1-EVLA	Eastvale MPD Line A (Mill-Cucamonga Creek)	-117.602032	33.967602
T1-EVLB	Eastvale MPD Line B (Mill-Cucamonga Creek)	-117.601892	33.960098
T1-EVLD	Eastvale MDP Line D (SAR Reach 3)	-117.579781	33.946701
T1-EVLE	Eastvale MDP Line E (SAR Reach 3)	-117.553434	33.950298
T1-MCSD	Magnolia Center SD (SAR Reach 3)	-117.415473	33.965599
T1-PHNX	Phoenix Storm Drain (SAR Reach 3)	-117.427128	33.963600
T1-SSCH	San Sevaine Channel	-117.506433	33.974300
T1-SNCH	Sunnyslope Channel	-117.427180	33.976200
T1-WLSD	Wilson Storm Drain (SAR Reach 4)	-117.372187	34.018700
San Bernardino County			
T1-SACH	San Antonio Channel @ SR 60	-117.72811	34.02470
T1-BRSC	Boys Republic South Channel @ confluence with Chino Creek	-117.72611	34.00208
T1-PPLN	Pipeline Ave 84" RCP outlet under bridge	-117.71506	33.98930
T1-CCCH	Carbon Canyon Creek @ Pipeline Ave	-117.71543	33.98620
T1-YRBA	Chino Creek, @ Yorba Ave ext., large outlet to SE of extension	-117.70192	33.98362
T1-LLSC	Lake Los Serranos Channel @ Red Barn Court crossing, above confluence with Chino Creek	-117.69106	33.97542
T1-CBLD	Chino Creek/San Antonio Creek @ ext. of Flowers St., behind Big League Dreams	-117.67493	33.95864
T1-CYP	Cypress Channel @ Kimball Avenue	-117.66039	33.96860
T1-CAPT	Cucamonga Creek @ Airport Drive	-117.60123	34.06294
T1-CNRW	Cucamonga Creek @ North Runway	-117.60072	34.05930
T1-CFRN	Cucamonga Creek @ Francis	-117.59848	34.04077
T1-WCUC	West Cucamonga Creek @ Cucamonga Creek	-117.59893	34.03257
T1-SR60	Cucamonga Creek @ above SR 60	-117.59929	34.03029
T1-CHRIS	Chris Basin Outflow @ Cucamonga Creek	-117.59906	34.00277
T1-CLCH	County Line Channel @ Cucamonga Creek	-117.60094	33.97431
T1-RISD	SW of Riverside Avenue @ SAR - City S.D.	-117.36447	34.02774
Los Angeles County			
CHINOCRK	Chino Creek upstream of San Antonio Channel	-117.73057	34.01343

¹ Coordinates are shown as Geographic WGS 1984 World Datum

Table 6-7. Prioritized Tier 1 drainage areas for Tier 2 source evaluation activities

Site ID	Jurisdictions	Drainage Acres	Human Presence ¹	MS4 Drainage Features
T1-EVLD	Eastvale	852	30%	Storm drains
T1-EVLE	Eastvale	798	100%	Storm drains
T1-CYP	Chino, Ontario	4,952	20%	Open channel with storm drain outfalls
T1-EVLB	Eastvale	334	80%	Storm drains
T1-ANZA	Riverside	7,313	20%	Open channel with storm drain outfalls
T1-CAPT	Ontario	1,050	40%	Storm drains
T1-CHRIS	Ontario	5,774	30%	Open channel with storm drain outfalls, culvert
T1-SSCH	Jurupa Valley, Fontana	3,337	40%	Open channel with storm drain outfalls
T1-EVLA	Eastvale	498	10%	Storm drains
CHINOCRK	Pomona, Claremont	6,032	30%	Storm drains
T1-PHNX	Riverside	503	10%	Storm drains
T1-CCCH	Chino Hills	3,934	0% ¹	Open channel with storm drain outfalls
T1-BRSC	Chino Hills	1,160	10%	Open channel with storm drain outfalls

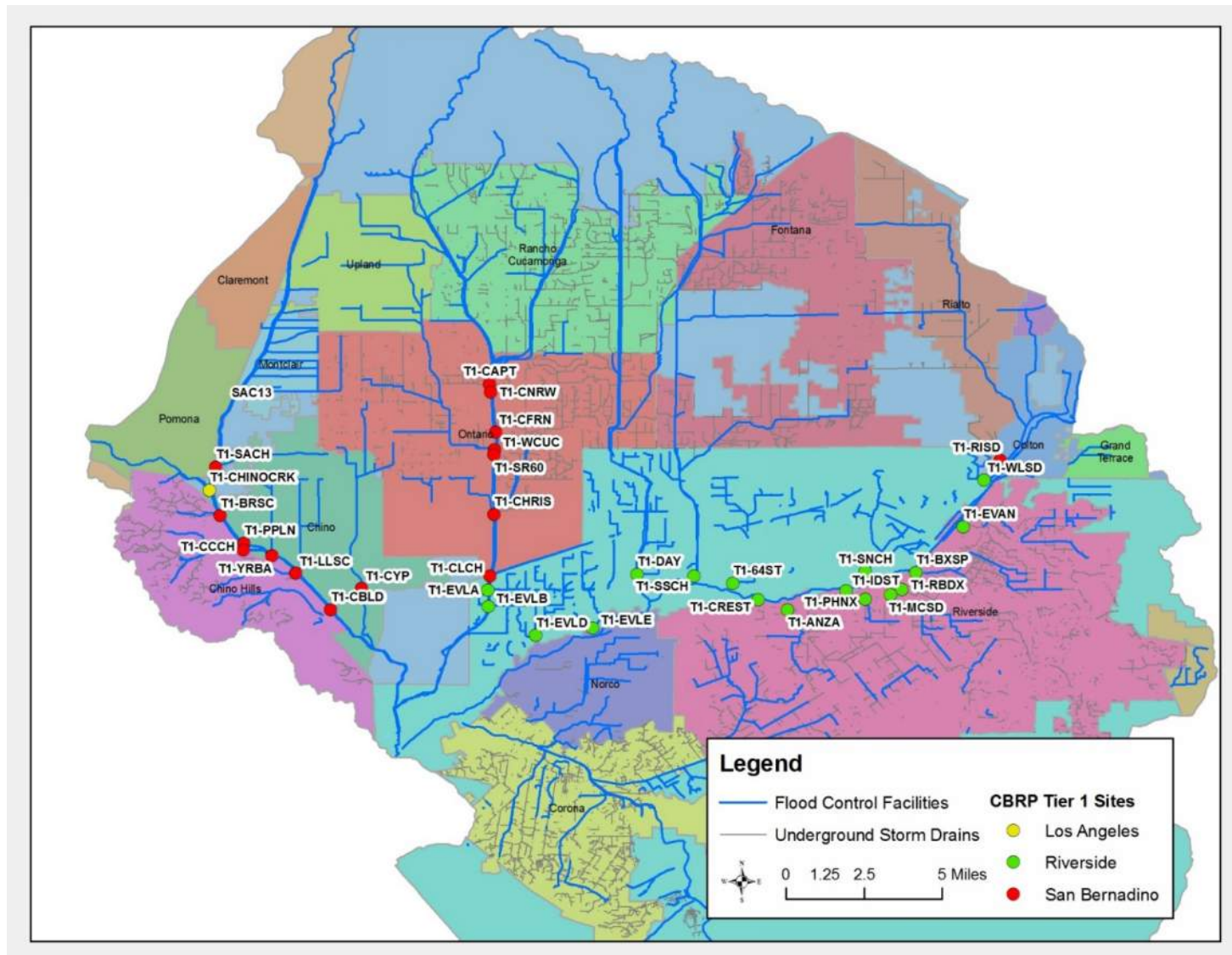


Figure 6-6. Tier 1 source evaluation Sites in the MSAR watershed to support implementation of the MSAR Bacteria TMDL

Broad-brush Approach

The broad-brush approach attempts to identify specific sources of human fecal bacteria by initially performing extensive field reconnaissance and screening investigations. These relatively low cost activities include field reconnaissance and deployment of secondary screening tools, and can be implemented at a large number of Tier 2 sites (see Section 11.4.2).

Results from field reconnaissance and secondary screening tool deployment are used to identify Tier 2 sites for bacterial water quality sample collection. On days when samples are collected from Tier 2 sites within the MS4s, samples are also collected from downstream Tier 1 sites to assess the relative role of the Tier 2 measurements in downstream bacteria characteristics.

The broad-brush approach provides a spatially robust dataset and has the potential to pinpoint specific management actions at an individual property scale to eliminate bacteria sources. MS4 permittees use results from field reconnaissance, secondary screening, and bacterial water quality analysis to guide implementation of short term management actions that address bacteria sources of concern. At the end of the dry season, a follow-up snapshot survey can be performed to determine the effectiveness of any management actions implemented.

The risk associated with this approach stems from the temporal variability in human *Bacteroides* detection, which was typically less than 40 percent of samples in the 2012 dry season at the downstream Tier 1 sites. Accordingly, there is a greater chance of missing the human fecal bacteria signal taking an approach with a single snapshot survey.

Subregional Approach

The subregional approach attempts to develop a better understanding of dry weather flow and water quality from subareas within the prioritized Tier 1 MS4 drainages. This approach involves weekly sample collection from the downstream Tier 1 site and at one or more major trunk confluences within the MS4 drainage system (Tier 2 sites). Samples are analyzed for *E. coli* over ten consecutive weeks to develop a baseline longitudinal characterization. Secondary screening tools are used in 5 of the 10 weeks to assess water quality at Tier 2 sites selected for source evaluation in neighborhood scale subareas upstream of each baseline bacterial water quality site. Field reconnaissance is important to identify the Tier 2 sites for baseline characterization in the initial weeks, and then to aid in selection of Tier 2 sites for source evaluation incorporating secondary screening tracer sample collection in the middle of the dry season. Lastly, in the latter portion of the dry season, samples are collected and analyzed for human *Bacteroides* at a subset of the Tier 2 sites based on information gathered from secondary screening and field reconnaissance. MS4 permittees use results from all phases of the source evaluation to guide implementation of short term management actions that address bacteria sources of concern.

The risk associated with this approach stems from the aggregation of large spatial areas, which may not provide the resolution needed to identify specific sources for focusing or targeting short-term management actions. However, since the Tier 2 source evaluations occur over two dry seasons, a subregional approach in the first year could be followed by adopting the broad-brush approach in smaller more manageable subareas in the second year of the program.

6.3.3 AgSEP Monitoring Program

6.3.3.1 Introduction

With EPA approval of the MSAR Bacteria TMDL in May 2007, agricultural dischargers (as defined by the TMDL) were required to complete specific implementation activities either in collaboration with other TMDL responsible parties or separately. Specifically, agricultural discharges were required to complete the following activities:

- Implement a watershed-wide compliance monitoring program (currently being implemented in collaboration with urban dischargers; see Section 6.3.1);
- Develop an AgSEP by November 30, 2007; and
- Develop a BASMP.

Agricultural Source Evaluation Plan

The purpose of the AgSEP was to identify specific activities, operations and processes in agricultural areas that contribute bacterial indicators to MSAR watershed waterbodies. The plan was to include a proposed schedule for the steps identified and include contingency provisions as needed to reflect any uncertainty in the proposed steps or schedule. Information from implementation of the AgSEP would be used by the Santa Ana Water Board and agricultural stakeholders to support development of the BASMP.

The AgSEP was submitted to the Santa Ana Water Board by November 30, 2007; it was approved on April 18, 2008³⁰. A component of the AgSEP involved implementation of an AgSEMP at key sites to gather bacterial indicator data. Monitoring was conducted during wet weather in the 2008-2009 wet season at four monitoring sites and included collection of field parameters, bacterial indicator data, and microbial source identification analyses (Table 6-8 and Figure 6-7). No additional sample collection from the AgSEP sample sites is currently planned. More details on the AgSEP program implementation are provided in Section 4.1.3.2 of the SAR Bacteria Monitoring Plan.

Bacterial Indicator Agricultural Source Management Plan

Per the MSAR Bacteria TMDL, the BASMP should include, plans and schedules for the following:

- Implementation of bacteria indicator controls, Best Management Practices (BMPs) and reduction strategies designed to meet load allocations;
- Evaluation of effectiveness of BMPs; and
- Development and implementation of compliance monitoring program(s).

A BASMP is currently under development by agricultural dischargers in the MSAR watershed. When complete it is expected to replace the AgSEP. Because this document is still under development, this section will be updated once the BASMP is finalized. Moreover, the final BASMP may include monitoring requirements designed to support implementation of the BASMP. If included in the final program, then these monitoring requirements will be incorporated into the SAR Bacteria Monitoring Plan and QAPP.

³⁰ Santa Ana Water Board Resolution: R8-2008-0044; April 18, 2008

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Table 6-8. AgSEMP monitoring sites

Site ID	Site Description	Longitude	Latitude
Prado Park Lake Drainage Area			
AG-G2	Grove Avenue Channel at Merrill Avenue	-117.37685	33.58986
AG-G1	Eucalyptus Avenue at Walker Avenue	-117.37163	33.59425
AG-E2	Euclid Avenue Channel at Pine Avenue	-117.38926	33.57220
Cucamonga Creek, Reach 1 Drainage Area			
AG-CL1	Eucalyptus Avenue at Cleveland Avenue (Backup to Walker Avenue, depending on flow conditions) (CL1)	-117.34031	33.59405
Chino Creek, Reach 1 Drainage Area			
AG-CYP1	Cypress Channel at Kimball Avenue (dual site; same as USEP site US-CYP)	-117.66043	33.96888

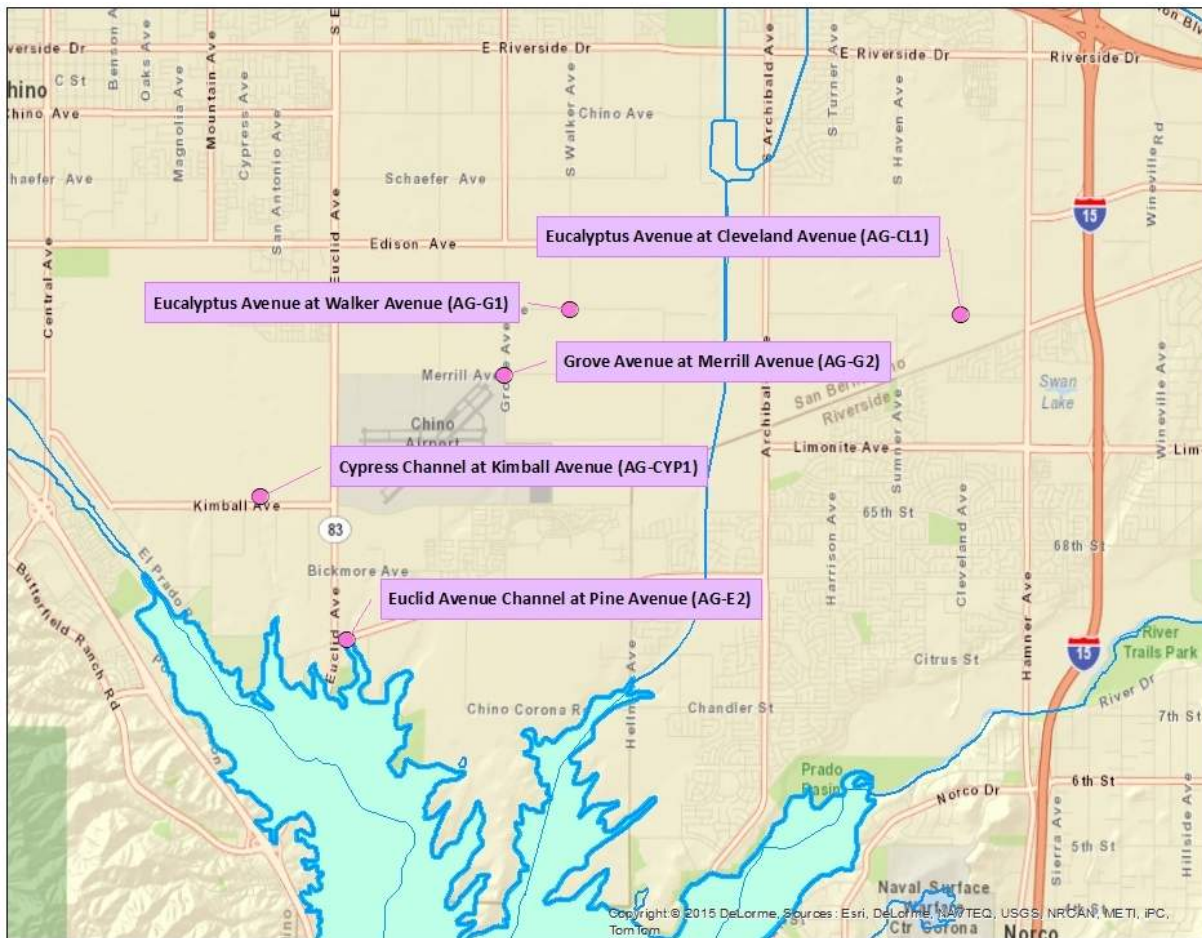


Figure 6-7. Location of AgSEMP sites sampled in 2008-2009.

6.4 Constituents to be Monitored and Measurement Techniques

The following water quality indicators will be measured at the Regional Monitoring Program (Section 6.2), watershed-wide wet weather (Section 6.3.1), Urban Source Evaluation (Section 6.3.2), and Agricultural Source Evaluation (Section 6.3.3.) monitoring sites, respectively.

6.4.1 Regional Monitoring Program Monitoring Sites

The following water quality indicators will be analyzed in water samples collected at each priority monitoring site on each sample date:

- *Field Analysis:* Temperature, conductivity, pH, dissolved oxygen, and turbidity will be measured with a Horiba multi-parameter probe or related instrument.
- *Flow:* During each time a site is sampled, if conditions are safe, flow will be characterized using a either a volumetric, cross-section velocity profile, or a visual estimate method.
- *Water Quality Analysis:* *E. coli*, and total suspended solids (TSS) concentrations in grab samples will be analyzed by a qualified laboratory for all Priority 1, 2 and 3 sites and all Priority 4 sites except P4-OC2 and P4-OC3. For P4-OC2 and P4-OC3, grab samples will be analyzed for *Enterococcus* and TSS

6.4.2 MSAR Bacteria TMDL Wet Weather Event Monitoring

Consistent with the MSAR Bacteria TMDL, the following water quality indicators will be analyzed in water samples collected at each site on each sample date:

- *Field Analysis:* Temperature, conductivity, pH, dissolved oxygen, and turbidity will be measured with a Horiba multi-parameter probe or related instrument.
- *Flow:* During each time a site is sampled, if conditions are safe, flow will be characterized using a either a volumetric, cross-section velocity profile, or a visual estimate method.
- *Water Quality Analysis:* *E. coli*, and TSS concentrations in grab samples will be analyzed by a qualified laboratory included in this QAPP (see appendices).

6.4.3 Urban Source Evaluation Monitoring Program

The following data will be collected when each Tier 1 or Tier 2 site is sampled:

- *Field Analysis:* Temperature
- *Water Quality Analysis:* *E. coli*, TSS, and *Bacteroides* analysis will be analyzed by a qualified laboratory included in this QAPP (see appendices).
- *Flow:* During each time a site is sampled, if conditions are safe, flow will be characterized using a volumetric, cross-section velocity profile, or visual estimate method
- *Bacteroides Analysis:* OCWD or qualified laboratory will use a semi-quantitative presence/absence method to analyze for human source *Bacteroides*.

In addition to measuring flow at Tier 1 sites, samplers assess the hydrologic connectivity of the surface flow at each site to the downstream impaired waterbody (Santa Ana River Reach 3, Mill Creek, Cucamonga Creek, and Chino Creek Reach 1 and 2) to evaluate if the tributary drain is actually discharging any runoff to the downstream waterbody. Under dry weather conditions, many Tier 1 locations, particularly along Santa Ana River Reach 3 are likely to not have hydrologic connectivity due to the long distance between Tier 1 discharge outfalls and the Santa Ana River. If there is no connection of surface waters, then the flow rate is assumed to be zero for that date only; collection of a water sample for laboratory analysis is optional, depending on the need for the data.

A variety of water quality screening tools can be effective at identifying specific MS4 sources of bacterial contamination with limited resources. These tools will be employed for Tier 2 bacteria source evaluation and are described in more detail in Section 11 of this QAPP:

- Ammonia Testing
- Potassium Testing
- Chlorine Test Strips
- Copper Test Strips
- Surfactant/Detergent Screening
- Canine scent tracking

6.4.4 Agricultural Source Evaluation Monitoring Program

The following data will be collected when each site is sampled:

- *Field Analysis:* Temperature, conductivity, pH, dissolved oxygen, and turbidity will be measured with a Horiba Multi-parameter probe or related instrument.
- *Water Quality Analysis:* *E. coli*, and TSS concentrations in grab samples will be analyzed by a qualified laboratory included in this QAPP (see appendices).
- *Flow:* During each time a site is sampled, if conditions are safe, flow will be characterized using a visual estimate method.
- *Bacteroides Analysis:* A qualified laboratory will assay water grab samples for *Bacteroides* host-specific markers for humans, ruminant, and domestic canine to determine if they are present and to provide a semi-quantitative estimate of their relative abundance.

6.5 Constraints to Monitoring

Under some circumstances, collection of water samples or field measurements may not be possible. For example, if flow in the channel is elevated, conditions may be too dangerous for taking a flow measurement by developing a cross section velocity profile. Another potential constraint would occur if the channel is dry, thus making it impossible to collect surface water samples. The field team will document any constraints in the field on the Field Data Forms. The data manager will incorporate

observational data from these site visits into the water quality database, indicating the reason why data were not collected at a given site.

6.6 Project Schedule

The project schedule is documented in in the SAR Bacteria Monitoring Plan and in Section 10 of this QAPP.

7. Quality Objectives and Criteria for Measurement Data

Table 7-1 summarizes the applicable data quality objectives for the types of measurements or analyses conducted under this project. Tables 7-2 and 7-3 summarize the specific data quality objectives for field measurements or constituents measured in the laboratory, respectively.

Table 7.1. Project data quality objectives

Measurement or Analyses Type	Applicable Data Quality Objective
Field Measurements	Accuracy, Precision, Completeness
Bacterial Analyses	Precision, Presence/Absence, Completeness
Water Quality Analyses, Surfactant Analyses	Accuracy, Precision, Recovery, Completeness

Accuracy will be determined by measuring one or more selected from performance testing samples or standard solutions from sources other than those used for calibration. Accuracy criteria for bacterial testing will be based on presence/absence testing rather than numerical limits owing to the difficulty in preparing solutions of known bacterial concentration.

Precision measurements will be determined on both field and laboratory replicates. The number of replicates for field measurements will be three, the number for TSS, ammonia, potassium, and surfactants will be two, and for bacterial testing, the number of replicates will be five.

Recovery measurements will be determined by laboratory spiking of a replicate sample with a known concentration of the analyte. The target level of addition is at least twice the original sample concentration and is applicable to TSS, ammonia, potassium, and surfactant analyses.

Completeness is the number of analyses generating useable data for each analysis divided by the number of samples collected for that analysis.

Method sensitivity is dealt with by the inclusion of the required SWAMP Target Reporting Limits, where such values exist. Target Reporting Limits exist for *E. coli*, *Enterococcus*, TSS, and ammonia.

No Target Reporting Limits were set for the potassium and surfactant laboratory analyses, or for the field analyses.

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Table 7-2. Data quality objectives for field measurements

Group	Parameter	Accuracy	Precision	Recovery	Target Reporting Limit	Completeness
Field Measurements	Conductivity	+/-5%	± 5%	NA	NA	90%
Field Measurements	Dissolved Oxygen	±0.5 milligrams/Liter (mg/L)	± 0.5 or 10%; whichever is greater	NA	NA	90%
Field Measurements	pH	+/- 0.5 units	± 0.5 or 5%, whichever is greater	NA	NA	90%
Field Measurements	Temperature	+/- 0.5°C	± 0.5 or 5%, whichever is greater	NA	NA	90%
Field Measurements	Turbidity	+/-10% or 0.1, whichever is greater	± 10% or 0.1, whichever is greater	NA	NA	90%
Field Measurements	Flow (visual estimate)	+/- 25% or 0.25, whichever is greater	± 25% or 0.25, whichever is greater	NA	NA	90%
Field Measurements	Flow (via flow instruments)	+/-10% or 0.1, whichever is greater	± 10% or 0.1, whichever is greater	NA	NA	90%
Field Measurements	Ammonia	+/-20%	+/-10%	NA	NA	90%
Field Measurements	Chlorine	+/-20%	+/-10%	NA	NA	90%
Field Measurements	Copper	+/-20%	+/-10%	NA	NA	90%
Field Measurements	Detergents/ Surfactants	+/-20%	+/-10%	NA	NA	90%
Field Measurements	Canine Scent Tracking	+/-20%	+/-10%	NA	NA	90%

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Table 7-3. Data quality objectives for laboratory measurements

Group	Parameter	Accuracy	Precision	Recovery	Target Reporting Limits	Completeness
Bacterial Analyses	<i>E. coli</i>	Positive results for target organisms. Negative results for non-target organisms	R _{log} within 3.27*mean R _{log} (reference is section 9020B 18th, 19th, or 20th editions of <i>Standard Methods</i>)	NA	10 colony forming units (cfu)/100 mL	90%
	<i>Enterococcus</i>	Positive results for target organisms. Negative results for non-target organisms	R _{log} within 3.27*mean R _{log} (reference is section 9020B 18th, 19th, or 20th editions of <i>Standard Methods</i>)	NA	10 colony forming units (cfu)/100 mL	90%
Bacteria Source Analyses	Genetic markers for human and canine (<i>Bacteroides thetaiotaomicron</i>), horse (<i>Bacteroides</i> spp.), bird (<i>Catelliboccus</i>), and rumen (<i>Prevotella</i>)	Positive results for target organisms. Negative results for results below detection limit of assay	NA	NA	10 cells/ 100 mL	90%
Conventional Constituents in Water	TSS	Standard Reference Materials (SRM, CRM, PT) within 95% CI stated by provider of material. If not available then with 80% to 120% of true value	Blind field duplicate and Laboratory duplicate, or MS/MSD 25% RPD	Matrix spike 80% - 120% or control limits at ± 3 standard deviations based on actual lab data, whichever is more stringent	1.0 mg/L	No SWAMP requirement; will use 90%
Nutrients in Water	Ammonia	Standard Reference Materials (SRM, CRM, PT) within 95% CI stated by provider of material. If not available then with 80% to 120% of true value	Blind field duplicate and Laboratory duplicate, or MS/MSD 25% RPD	Matrix spike 80% - 120% or control limits at ± 3 standard deviations based on actual lab data, whichever is more stringent	0.1 mg/L	No SWAMP requirement; will use 90%
Inorganic Analytes in Water	Potassium	Standard Reference Materials (SRM, CRM, PT) within 95% CI stated by provider of material. If not available then with 80% to 120% of true value	Blind field duplicate and Laboratory duplicate, or MS/MSD 25% RPD	Matrix spike 80% - 120% or control limits at ± 3 standard deviations based on actual lab data, whichever is more stringent	NA	No SWAMP requirement; will use 90%
Detergents/ Surfactants in Water	MBAS	Standard Reference Materials (SRM, CRM, PT) within 95% CI stated by provider of material. If not available then with 80% to 120% of true value	Blind field duplicate and Laboratory duplicate, or MS/MSD 25% RPD	Matrix spike 80% - 120% or control limits at ± 3 standard deviations based on actual lab data, whichever is more stringent	NA	No SWAMP requirement; will use 90%

8. Special Training Needs/Certification

All persons involved in the field sampling activities to implement the SAR Bacteria Monitoring Plan will be trained prior to any field sampling. Training will take place to ensure that sampling field members are familiar with the protocols and sampling sites.

All individuals that participate in sampling activities are required to have attended (at a minimum) the “4-hour Basic Site Safety Training” provided by an appropriately qualified trainer and/or contractor of the Health and Safety branch of the State, and/or equivalent university training. The training will cover the general health and safety issues associated with fieldwork, including sampling. The Project Manager for each Responsible Agency will provide specific training, pertinent to the details of a particular sampling program. This training will include, but not be limited to, proper use of field equipment, health and safety protocols, sample handling protocols, and chain of custody protocols.

Field staff training is documented and filed at the office of the Project Manager for each Responsible Agency. Documentation consists of a record of the training date, instructor, whether initial or refresher, and whether the course was completed satisfactorily.

All commercial laboratories will provide appropriate training to its staff as part of its Standard Operating Procedure. All laboratories will maintain their own records of its training that comply with OSHA requirements. Those records can be obtained, if needed, from each contract laboratory through their Quality Assurance Officer.

9. Documents and Records

The following documentation and records procedures will be followed (Table 9-1):

- A Final Annual Report will be submitted electronically to the Santa Ana Water Board by June 30th of each year to document the findings from the previous sample year.³¹ Electronic copies will be provided to each Responsible Agency. The first Final Annual Report will be submitted by June 30, 2017.
- Each Responsible Agency's Project Manager will maintain a record of all field data collection activities and samples collected and analyzed. All samples delivered to contract laboratories for analysis will include completed Field COC forms (Attachment E). Upon request, all contracted laboratories will generate records for sample receipt and storage, analyses, and reporting.
- Contract laboratories will submit the results of all laboratory analyses to the Responsible Agency Monitoring Manager that submitted the samples for analysis. Field data collected by each Responsible Agency will be maintained onsite and uploaded into a spreadsheet/database while sampling is ongoing within a sample year. The spreadsheet/database format will be provided to all Responsible Agency Project Managers by the Project Director.
- For each sample year, electronic records of field data and laboratory sample results, copies of COC and original field data sheets and flow measurement forms for sites where a velocity cross section profile method was used to measure flow will be kept on file by the Responsible Agency. By January 15th of each reporting year, all forms, data sheets, or electronic files associated with non-wet weather event sampling will be provided to the Project Director to support preparation of the Annual Report. Within 15 days after completion of wet weather event sampling, all forms, data sheets, or electronic files associated with the sampling event will be provided to the Project Director to support preparation of the Annual Report.
- Contract laboratories will maintain electronic or paper records pertinent to the implementation of the SAR Bacteria Monitoring Plan at the laboratory's main office for at least three years. By January 15th of each year, each contract laboratory will provide to the Project Director a QA/QC Report that assesses compliance with laboratory QA/QC protocols for dry weather samples processed during the previous sample year (generally May 1 through November 30). In addition, by April 15th of each year, each contract laboratory will provide to the Project Director a QA/QC Report that assesses compliance with laboratory QA/QC protocols for wet weather event samples processed during the previous sample year during the wet season (between November 1 and March 31). At any time, copies of records or QA/QC reports held by the contract laboratories will be provided to a Responsible Agency Project Manager (or Project QA Officer) or Project Director upon request.
- Each Responsible Agency's Data Manager will manage field and laboratory data results by ensuring that all such data are uploaded into a database or spreadsheet template provided by the Project

³¹ A sample year is the period from May 1 through April 30 and includes the following sample activity: (a) collection of dry weather samples from Priority 1, 2, 3, and 4 sites from May through September; (b) collection of dry weather samples from Priority 1, 2 and 3 sites in late October through November; and (c) collection of samples from one wet weather event in the MSAR watershed between November 1 and March 31. See Section 10 for specific sample schedules for each priority site.

Director. By January 15th of each year, each Responsible Agency Project Manager will submit to the Project Director the database or spreadsheet file containing the previous sample year's field and laboratory data for dry weather samples collected during the previous sample year (generally May 1 through November 30). In addition, by April 15th of each year, each Responsible Agency Project Manager will submit to the Project Director the database or spreadsheet file containing the previous sample year's field and laboratory data for wet weather event samples collected during the previous sample year during the wet season (between November 1 and March 31),.

- As part of the preparation of each Annual Report, the Project Director will ensure that all field data and laboratory data results (including QA/QC data) from each Responsible Agency are combined and uploaded to CEDEN. Data will be uploaded no later than 30 days after submittal of the Final Annual Report to the Santa Ana Water Board.
- Copies of this QAPP will be distributed to all Responsible Agencies involved with the SAR Bacteria Monitoring Program. Copies will be sent to each Contract Laboratory QA Officer for distribution to appropriate Laboratory Personnel. Any future amended QAPPs will be held and distributed in the same manner. All originals of this QAPP and its amendments will be held by the Project Director. Copies of versions, other than the most current, will be discarded so as not to create confusion.
- The Project Director will prepare a Draft Annual Report by April 30th of each year to reflect findings from sampling conducted during the previous sample year. This report will include findings from (a) all RMP sites; and (b) any required TMDL monitoring activities conducted to support implementation of a bacteria TMDL, e.g., wet weather sampling. After providing an opportunity for review of the Draft Annual Report and revising the draft report based on comments received, a Final Annual Report will be submitted electronically to the Santa Ana Water Board by June 30 of each year.
- At a minimum, the Final Annual Report will be electronically distributed to each Responsible Agency and the Santa Ana Water Board. The Final Annual Report will be made available to the public on either the Santa Ana Water Board or Project Director's website.

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Table 9-1. Record retention, archival, and disposition information

Record Type	Document Type	Retention	Archival	Disposition
Sample Collection Records	Field Logs	Responsible Agency during sample year	Project Director	Project Director
Analytical Records	Laboratory results	Responsible Agency and Contract Laboratories during sample year	Project Director	Project Director
	COC Forms	Responsible Agency and Contract Laboratories during sample year	Project Director	Project Director
Assessment Reports	QA/QC Updates	Responsible Agency during sample year	Project Director	Project Director
	QA/QC Final Report	Responsible Agency during sample year	Project Director	Project Director
	Field Sampling Review	Responsible Agency Project QA Officer during sample year	Project Director	Project Director
	Internal Technical Audit of Database Management	Responsible Agency Data Manager during sample year	Project Director	Project Director
Reports	Santa Ana River Bacteria Monitoring Program Annual Report	Responsible Agency Project Manager	Project Director	Project Director and Santa Ana Water Board

Group B: Data Generation and Acquisition

10. Sampling Process Design

10.1 Regional Monitoring Program

For dry weather monitoring activities at RMP Sites (see Section 6.2), the sampling effort is generally described as follows (see Tables 10-1 and 10-2):

- *Priority 1 and 2 Sites*: Priority 1 and 2 sample sites will be sampled during dry weather (defined as no measurable rainfall within a 72 hour period prior to sampling) for a 20-week period during the warmest part of the year between May 1 and September 30. In addition, Priority 1 sample sites will also sampled during one 5-week period from end of October through most of November each year during the cooler season. The resulting dataset will include 25 samples each year from each site and provide sufficient data to calculate 16 geometric means during the 20-week sample period and one geometric mean during the cool season. Table 10-1 provides a sampling schedule from January 1, 2016 through 2020.
- *Priority 3 Sites, (five-week sample events rotated on annual basis)*: Fourteen monitoring sites are included in this Priority category. These sites have been grouped, generally by location, into five groups (Table 10-2). The goal is to collect five samples over a five week consecutive period during dry weather once each year. Accordingly, grouped sites will be sampled during dry weather (defined as no measurable rainfall within a 72 hour period prior to sampling) on a rotational basis over a period of a year so that all sites are sampled at least once each year (Table 10-2). The overall sample schedule for these sites overlaps with the Priority 1 & 2 sample site schedule to maximize efficiency with the collection of samples. In the first year of implementation, Groups 1 through 5 will be sampled in order over a one year period. In subsequent years, the order of groups varies so that a Group's assigned five-week sample period varies by season over the long-term (e.g., summer vs. fall or winter).
- *Priority 4 Sites, (once per year)*: Water quality samples will be collected during dry weather once per year and analyzed for *E. coli* or *Enterococcus* to determine if the result exceeds the antidegradation target threshold value for the site (equal to the 75th percentile of the lognormal distribution fitted to historical data). If an exceedance of the antidegradation target is observed, additional *E. coli* or *Enterococcus* samples will be collected once/month for the three following months. If any of the follow-up samples exceed the antidegradation target, then sampling will continue on a monthly basis until source(s) of the increased bacterial indicator concentration is identified and mitigated and bacterial indicator levels return to below the antidegradation target in three of four samples collected over three consecutive months. The annual dry weather sample will be collected during the summer season between June 21 and September 21 when REC2 activities are most likely to occur. If additional sampling is required due to an observed exceedance, the schedule will be determined based on the process described above.

10.2 TMDL Monitoring Programs

10.2.1 MSAR Bacteria TMDL Wet Weather Event Monitoring

One wet weather event is targeted for sampling each wet season, defined as November 1 through March 31 in the MSAR Bacteria TMDL. The goal of wet weather event sampling is to collect bacterial indicator

data during the rising and falling limbs of the hydrograph. To accomplish this goal, a wet weather sample event requires the collection of four samples over an approximately four day period:

- Sample 1 – Target sample collection on the day of the storm event when it is apparent that flow within the channel is elevated above typical dry weather conditions as a result of rainfall induced runoff.
- Sample 2 – Collect samples approximately 48 hours after collection of Sample 1.
- Sample 3 – Collect samples approximately 72 hours after collection of Sample 1.
- Sample 4 – Collect samples approximately 96 hours after collection of Sample 1.

10.2.2 Urban Source Evaluation Monitoring Program

Tier 1 and 2 source evaluation activities contained in the CBRP schedule were completed during the period from 2012 to 2014. Additional site-specific monitoring activities are ongoing where needed to answer local questions. Any water quality samples collected as part of these activities are conducted according to the requirements of this QAPP.

10.2.3 Agricultural Source Evaluation Monitoring Program

Prior agricultural source evaluation monitoring occurred in 2008-2009. A BASMP is currently under development by agricultural dischargers in the MSAR watershed. The final BASMP may include monitoring requirements designed to support implementation of the management plan. If included in the final program, then the approach and schedule will be added to Section 10 of this QAPP.

Table 10-1. Sample schedule for Priority 1 and 2 waters during dry weather conditions (2016 - 2020)

Year	Sample Season	First Week of Sampling	Last Week of Sampling	Priority 1 Waters	Priority 2 Waters
2016	Warm Season	May 8	September 18	All Table 3.1 Waters	All Table 3.3 Waters
	Cool Season	October 30	November 27	All Table 3.1 Waters	All Table 3.3 Waters
2017	Warm Season	May 7	September 17	All Table 3.1 Waters	All Table 3.3 Waters
	Cool Season	October 29	November 26	All Table 3.1 Waters	All Table 3.3 Waters
2018	Warm Season	May 6	September 16	All Table 3.1 Waters	All Table 3.3 Waters
	Cool Season	October 28	November 25	All Table 3.1 Waters	All Table 3.3 Waters
2019	Warm Season	May 5	September 15	All Table 3.1 Waters	All Table 3.3 Waters
	Cool Season	October 27	November 24	All Table 3.1 Waters	All Table 3.3 Waters
2020	Warm Season	May 10	September 20	All Table 3.1 Waters	All Table 3.3 Waters
	Cool Season	October 25	November 22	All Table 3.1 Waters	All Table 3.3 Waters

Table 10-2. Sample schedule for Priority 3 waters during dry weather conditions (2016 - 2020)

Year	First Week of Sampling	Last Week of Sampling	Priority 3 Waters
2016	May 8	June 5	Group 1: Bolsa Chica Channel, , Santa Ana River Reach 2
	June 12	July 10	Group 2: Peters Canyon Wash, San Diego Creek Reach 1, San Diego Creek Reach 2, Borrego Creek, Serrano Creek

Table 10-2. Sample schedule for Priority 3 waters during dry weather conditions (2016 - 2020)

Year	First Week of Sampling	Last Week of Sampling	Priority 3 Waters
	July 17	August 14	Group 3: Los Trancos Creek, Morning Canyon Creek, Buck Gully Creek
	August 21	September 18	Group 4: Santa Ana Reach 4
	October 30	November 27	Group 5: Goldenstar Creek, Lake Fulmor
2017	May 7	June 4	Group 2: Peters Canyon Wash, San Diego Creek Reach 1, San Diego Creek Reach 2, Borrego Creek, Serrano Creek
	June 11	July 9	Group 3: Los Trancos Creek, Morning Canyon Creek, Buck Gully Creek
	July 16	August 13	Group 4: Santa Ana Reach 4
	August 20	September 17	Group 5: Goldenstar Creek, Lake Fulmor
	October 29	November 26	Group 1: Bolsa Chica Channel, , Santa Ana River Reach 2
2018	May 6	June 3	Group 3: Los Trancos Creek, Morning Canyon Creek, Buck Gully Creek
	June 10	July 8	Group 4: Santa Ana Reach 4
	July 15	August 12	Group 5: Goldenstar Creek, Lake Fulmor
	August 19	September 16	Group 1: Bolsa Chica Channel, , Santa Ana River Reach 2
	October 28	November 25	Group 2: Peters Canyon Wash, San Diego Creek Reach 1, San Diego Creek Reach 2, Borrego Creek, Serrano Creek
2019	May 5	June 2	Group 4: Santa Ana Reach 4
	June 9	July 7	Group 5: Goldenstar Creek, Lake Fulmor
	July 14	August 11	Group 1: Bolsa Chica Channel, , Santa Ana River Reach 2
	August 18	September 15	Group 2: Peters Canyon Wash, San Diego Creek Reach 1, San Diego Creek Reach 2, Borrego Creek, Serrano Creek
	October 27	November 24	Group 3: Los Trancos Creek, Morning Canyon Creek, Buck Gully Creek
2020	May 10	June 7	Group 5: Goldenstar Creek, Lake Fulmor
	June 14	July 12	Group 1: Bolsa Chica Channel, , Santa Ana River Reach 2
	July 19	August 16	Group 2: Peters Canyon Wash, San Diego Creek Reach 1, San Diego Creek Reach 2, Borrego Creek, Serrano Creek
	August 23	September 20	Group 3: Los Trancos Creek, Morning Canyon Creek, Buck Gully Creek
	October 25	November 22	Group 4: Santa Ana Reach 4

11. Sampling Methods

11.1 Sample Collection

Dry weather sampling at priority sites should only occur under dry weather conditions defined as no measurable rainfall within a 72 hour period prior to sampling. During dry weather conditions, if flow is elevated due to non-wet weather sources, e.g., upstream dam releases or dewatering activities, sample collection should still occur as long as conditions are safe. The elevated water levels will be documented on the field data sheet and flow will be estimated (see Section 11.3).

11.1.1 Water Samples

In-stream sampling consists of grab samples collected approximately mid-stream and at the water surface during designated sample activities following sampling methods provided below. Water samples are best collected before any other work is done at the site. If other work is done prior to the collection of water samples (for example, flow measurements or other field measurements), bottom sediment may be disturbed into the water column, which may not reflect representative conditions for water chemistry and bacteria analyses. Wading by sample collection staff shall not occur during collection of samples for bacterial and TSS analyses.

To the extent practical, water samples are collected from a location in the stream (or storm drain as may be the case for urban or agricultural source evaluation activities) where the stream visually appears to be well-mixed and flowing. Ideally this would be at the centroid of the flow (*Centroid* is defined as the midpoint of that portion of the stream width that contains 50% of the total flow), but depth and flow do not always allow collection of samples from the centroid location. Ultimately, the selection of the best location to collect water samples is based on best professional judgment. In addition, the sample should be collected in an area free of debris or algae. Samples shall not be collected if conditions are determined to be unsafe during an on-site assessment by the field team leader. Photo documentation shall be provided to illustrate unsafe conditions and the specific issues of concern shall be noted on the field form.

For sites where the samples will be taken from a distance, a sampling pole will be used. This sampling pole is approximately 7 feet long and has a mechanism that holds the sample bottle in place. The mechanism should be sterilized in the field with a 70 percent ethanol solution prior to the collection of each sample. After being cleaned with ethanol (70%) the sampling pole should be rinsed thoroughly. Allow the pole to air-dry before the sample is taken. A similar sampling pole that extends to a greater height may be used for sites where sampling from a bridge is necessary.

Table 11-1 summarizes information relevant to sample collection. The following text lists steps to take when collecting a water sample, (including, but not limited to steps from *EPA's Volunteer Stream Monitoring: A Methods Monitoring Manual*, EPA 841-B-97-003, November 1997):

- (1) Label each sample container with a site identification number (Site ID), sample identification number (Sample ID), analysis information, project identification number (Project ID), date, and time (ideally, some of this information may be pre-labeled on the containers). After sampling, if waterproof labels are not used, secure the label by taping it around the bottle with clear packaging tape.

- (2) For *E. coli* and *Enterococcus* samples the sterilized bottle will contain sodium thiosulfate for chlorine elimination. For ammonia and potassium samples, the bottles will contain sulfuric acid and nitric acid for preservation, respectively. Therefore, the bottles for analysis of these constituents cannot be held under the water to collect a sample. In contrast, the sterilized TSS bottle contains no preservatives and no such restrictions exist.
- (3) When wading (if applicable) to the sampling point, try not to disturb bottom sediment before collection of a sample.
 - a. To collect a water sample with a bottle containing a preservative, stand in the water facing upstream. Open the lid carefully; at all times, avoid touching the inside of the bottle or cap. If you accidentally touch the inside of the bottle or cap, use another bottle. The sample should be collected from the surface from your upstream side, i.e., in front of you, by holding the bottle at an angle so that the preservative does not flow out and sample does not overflow the bottle. Fill the bacteria bottle to the 100 or 125 mL mark. Do not overfill the sample bottles (so the sample can be shaken before analysis). Recap the bottle, remembering not to touch the inside.
 - b. To collect a water sample with a bottle without preservative, stand in the water facing upstream. The sample should be collected from the surface from your upstream side, i.e., in front of you, by holding the bottle upright under the surface while it is capped. Open the lid carefully to let the water run in. At all times, avoid touching the inside of the bottle or cap. If you accidentally touch the inside of the bottle or cap, use another bottle. Once the bottle is filled, recap the bottle, remembering not to touch the inside.
 - c. An alternative approach to (3).a and (3).b above is to use a separate sterilized bottle to collect a water sample to transfer to the sample containers with or without preservatives. If using a sterilized transfer vessel for both TSS and *E. coli* samples, water can then be decanted from this bottle (after shaking the sample) into the sample containers that will be submitted to the laboratory.
- (4) When flow is too shallow to collect a surface sample, such as when there is sheet flow across a channel, the sample should be collected at a location where there is greater water depth, such as at a seam in the channel, or near an obstruction, or where the flow spills over a concrete apron or lip. Follow the sample collection procedures for bottles with and without preservative as described above.

If there are no features in the channel that increase water depth and it is not possible to fill the bottle directly from the flow, then carefully collect a sample as follows (adapted from *Standard Operating Procedure for the Collection of Bacteria Samples from Storm Drains and Receiving Waters (Creeks, lagoons, bays, and ocean) for the City of San Diego 2002-03 Coastal Monitoring Annual Report*):

Use a clean, sterile syringe to collect a water sample from the surface without sampling floating particulates, yet far enough away from the bottom to avoid suction of soil, silt, and organic matter. Care should be taken to not touch the tip of the syringe. Draw back the plunger slowly while monitoring the syringe for organic matter, silt, sand, and floating particulates. Without touching the syringe to the sample bottle, dispense the sample into the sample bottle. Repeat until the sample bottle is full. Appropriately discard used syringe after each sample.

- (5) Place the bottles in a cooler with cold packs for transport to the laboratory. The maximum holding time prior to water quality analysis for bacteria concentrations is 6 hours; the maximum holding time prior to *Bacteroides* analysis is 24 hours. Bottles will be provided by the laboratories for each sample and depending on the water quality analyses required may include:
- (a) Water quality analysis laboratory – A single 100 to 125 mL bottle for *E. coli*, one 1,000 mL bottle for TSS, one 500 mL bottle for surfactants, a single 500 mL bottle for potassium, and a 100 mL bottle for ammonia
 - (b) OCWD – One 1,000 mL bottle for *Bacteroides* analysis
- (6) Field QA Samples:
- (a) *Field Equipment Blanks*
 - (i) *Regional Monitoring Program and TMDL Program Monitoring (wet weather and Tier 1)* - One set of field equipment blank samples (equal volume for each constituent) will be included for each sample event.
 - Sterile deionized (DI) water is poured through any equipment used to collect *E. coli* or *Enterococcus* samples at the site where the field equipment blank is being collected and then into the 100 or 125 mL *E. coli* sample containers.
 - For the *Bacteroides* equipment blanks, high purity water (in amber bottles) from an approved laboratory will be poured into the 1 liter sample bottle.
 - For the TSS field equipment blank, distilled water is poured through any equipment used to collect the TSS sample at the site where the field equipment blank is being collected and then into the 1 liter TSS sample bottle. If no equipment is used to collect the TSS sample, then the distilled water is poured directly into the 1 liter TSS sample bottle.
 - One set of field equipment blank samples will be collected for each sample event (one sample event encompasses all samples collected within a given week); the site for collection of blank samples will be selected on a rotational basis. After field equipment blanks have been collected from all monitoring sites, the rotation will start again with the first monitoring site.
 - (ii) *Urban Source Evaluation Program Tier 2 Monitoring* – No field equipment blanks are collected.
 - (b) *Field Replicates* – Field replicates are taken by collecting two sets of samples at the same location within five minutes of each other. Field replicates are collected as follows:
 - (i) *Regional Monitoring Program Sites* – One set of field replicates will be collected for each sample event (one sample event encompasses all samples collected within a given week) conducted at Priority 1, 2, 3 or 4 sites. The site for collection of replicate samples will be selected on a rotational basis (if more than one site sampled during a sample event. After

replicates have been collected from all monitoring sites, the rotation will start again with the first monitoring site. .

(ii) *MSAR Bacteria TMDL Wet Weather Event* – During the four day sample event, replicates are collected from one site on one of the sample days. Site is randomly selected.

(iii) *Urban Source Evaluation Program Tier 2 Monitoring* - No replicate samples are collected.

11.1.2 Sediment and Biofilm Samples

Sampling of sediment or biofilms may occur as part of Tier 1 or Tier 2 sampling events (see Section 6.3.2.2) to support TMDL-related source evaluation activities. Surface sediment and biofilm grab samples will be collected from the midpoint of shallow, wadable channel and stream widths. When multiple samples are collected along a transect of the stream, sample locations should reflect 25 percent, 50 percent, and 75 percent of the stream width. In cases where both water and samples are collected from the same study site, water should be collected first and care should be taken to not disturb the sediment.

The following lists contain specific steps to take when collecting a sediment sample (adapted from *EPA's Field Sampling Guidance Document #1215 for Sediment Sampling*, September 1999):

- (1) Label each container with Site ID, Sample ID, analysis information, Project ID, date, and time (some of this information may be pre-labeled on the containers). After sampling, secure the label by taping it around the bottle with clear packaging tape.
- (2) When wading (if applicable) to the sampling point, do not disturb bottom sediment.
- (3) Stand in the water, facing upstream. Collect the sediment and biofilm sample on your upstream side, i.e., in front of you.
- (4) Use a sterile stainless steel or plastic scoop or similar equipment type to scoop sediment along the bottom of the waterbody surface in the upstream direction. For biofilms, scoop along the surface the biofilms are attached to. Do not use plated scoops (e.g., garden spades) as they can result in contamination of samples.
- (5) Decant excess water without loss of fine particles from the scoop and deposit sediment into sterile sample container. Avoid touching the inside of the bottle or cap with anything but sample material. If you accidentally touch the inside, use another bottle. Fill the bottle leaving a 1-inch air space.
- (6) Carefully recap the bottle without touching the inside of the container.
- (7) Place the bottles in a cooler with cold packs for transport to the laboratory. The maximum holding time prior to water quality analysis for *E. coli* bacteria concentrations is 6 hours; the maximum holding time prior to *Bacteroides* analysis is 24 hours. Bottles will include a single, sterile 50 mL tube for both *E. coli* and bacterial indicator source analyses.
- (8) Field QA Samples

- (a) *Field Blanks* – One set of field equipment blanks will be included for each sample event (one sample event encompasses all samples collected within a given week). The site for collection of blank samples will be selected on a rotational basis. After blanks have been collected from all monitoring sites, the rotation will start again with the first monitoring site. To collect the sample, sterile deionized water is poured through any equipment used to collect samples at the site where the field equipment blank is being collected and then into the respective sample containers for each constituent.
- (b) *Field Replicates* – Field replicates are taken by collecting two sets of samples at the same location within five minutes of each other. Field replicates will be collected from at least one sample site per sample event (one sample event encompasses all samples collected within a given week). The site for collection of replicate samples will be selected on a rotational basis. After replicates have been collected from all monitoring sites, the rotation will start again with the first monitoring site.

11.2 Field Measurements

Field measurements are made at all monitoring sites except Tier 2 sites. For Tier 2 sites, field measurements will be made on an as needed basis where necessary to support the purposes of monitoring activities at these sites.

After collecting the water samples, record the water temperature, pH, conductivity, turbidity, and dissolved oxygen concentration. These parameters as well as other field data are measured and recorded using a multi-parameter probe. When field measurements are made with a multi-parameter instrument, sufficient time should be allowed for the instrument to equilibrate in the water before field measurements are recorded.

Field measurements are made at the centroid of surface flow if the stream visually appears to be completely mixed from shore to shore. For routine field measurements, the date, time and depth are reported as a grab. Below is a brief discussion of each field parameter to be measured:

- *Dissolved Oxygen* – Calibrate the dissolved oxygen sensor on the multi-probe instrument at the beginning of each day of field measurements. Preferably, dissolved oxygen is measured directly in-stream close to the flow centroid. The dissolved oxygen probe must equilibrate for at least 90 seconds before dissolved oxygen is recorded to the nearest 0.1 mg/L. Since dissolved oxygen takes the longest to stabilize, record this parameter after temperature, conductivity, turbidity, and pH.
- *pH* – Preferably, specific conductance is measured directly in-stream close to the surface flow centroid. If the pH meter value does not stabilize in several minutes, out-gassing of carbon dioxide or hydrogen sulfide or the settling of charged clay particles may be occurring. If out-gassing is suspected as the cause of meter drift, collect a fresh sample, immerse the pH probe and read pH at one minute. If suspended clay particles are the suspected cause of meter drift, allow the sample to settle for 10 minutes, and then read the pH in the upper layer of sample without agitating the sample. With care, pH measurements should be accurately measured to the nearest 0.1 pH unit.
- *Conductivity* – Preferably, specific conductance is measured directly in-stream close to the surface flow centroid. Allow the conductivity probe to equilibrate for at least one minute before specific conductance is recorded to three significant figures (if the value exceeds 100 $\mu\text{S}/\text{cm}$). The primary

physical problem in using a specific conductance meter is entrapment of air in the conductivity probe chambers. The presence of air in the probe is indicated by unstable specific conductance values fluctuating up to $\pm 100 \mu\text{S}/\text{cm}$. The entrainment of air can be minimized by slowly, carefully placing the probe into the water; and when the probe is completely submerged, quickly move it through the water to release any air bubbles.

- *Temperature* - Temperature is measured directly in-stream close to the surface flow centroid. Measure temperature directly from the stream by immersing a multi-parameter instrument.
- *Turbidity* - Turbidity is measured directly in-stream close to the surface flow centroid. Measure turbidity directly from the stream by immersing a multi-parameter instrument or use of a Hach turbidimeter.

11.3 Instantaneous Flow Monitoring

With one exception, flow measurements will be recorded by field personnel for every site visited using one of the methods described below. The exception is monitoring sites near a stream gage station that provides representative flow data for the monitoring site. The data from the gage station may be used instead of estimating flow in the field.

11.3.1 Visual Flow Estimate

Flow estimate data may be recorded for a non-tidally influenced stream when it is not possible to measure flows by the volumetric or cross section velocity profile methods described above either because flows are too high or so shallow that obtaining a velocity measurement is difficult or impossible. Visual flow estimates are subjective measures based on field personnel's experience and ability to estimate distances, depths, and velocities.

- (1) Observe the stream and choose a reach of the stream where it is possible to estimate the stream cross section and velocity. Estimate stream width (feet) at that reach and record.
- (2) Estimate average stream depth (feet) at that reach and record.
- (3) Estimate stream velocity (ft/s) at that reach and record. A good way to do this is to time the travel of a piece of floating debris. This can be done by selecting points of reference along the stream channel which can be used as upper and lower boundaries for an area of measurement. After establishing the boundaries, measure the length of the flow reach. One person stands at the upper end of the reach and drops a floating object and says "start." A second person stands at the lower end of the reach and times the number of seconds for the floating object to float the reach. This measurement is conducted three times and the three results are averaged. The velocity is the length of the reach in feet divided by the average time in seconds.
- (4) If doing this method from a bridge (for example, because flows are too high to be in the channel), measure the width of the bridge. Have one person drop a floating object (something that can be distinguished from other floating material) at the upstream side of the bridge and say "start". The person on the downstream side of the bridge will stop the clock when the floating object reaches the downstream side of the bridge. Divide the bridge width by the number of seconds to calculate the velocity. The velocity should be measured at multiple locations along the bridge at least three times. These velocities are averaged.

- (5) Multiply stream width (feet) by average stream depth (feet) to determine the cross sectional area (ft^2) which when multiplied by the stream velocity (ft/s) and a correction constant, gives an estimated flow (ft^3/s).

11.3.2 Measured Flow Estimate

Where possible, volumetric measurements will be collected according to the following procedures:

- *Volumetric Flow (Q) Estimate* - Where possible, a volumetric flow measurement approach will be used. This method shall not be used if conditions are determined to be unsafe by an on-site assessment by the field team leader. A volumetric flow measurement entails estimation of the time in seconds (t) required to fill a 5 gallon bucket with concentrated runoff. Sites with low flow and a free outfall would allow for this type of flow measurement. The following equation would then give the flow rate for a test with one 5-gallon bucket of volume captured, Q (cfs or ft^3/sec) = $0.67 * t$. If there are multiple points where runoff is concentrated, then volumetric measurements can be made at each point along the stream and summed to provide total discharge.

If volumetric measurements is not feasible at a site, then a depth-velocity estimate will be developed according to the cross-section velocity profile procedures.

- *Cross-Section Velocity Profile Flow Measurement* - The following steps guide the development of a velocity profile for a streamflow cross section. This approach will require that the field personnel be equipped with a Marsh-McBirney flow meter or equivalent, top-setting wading rod (preferably measured in tenths of feet), and a tape measure (with gradations every tenth of a foot). The following procedure is used to collect data:

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Table 11-1. Sample collection for field measurements (see discussion in QAPP Section 10 or Monitoring Plan)

Sampling Location	Site ID Number	Matrix	Depth (units)	Analytical Parameter	No. Samples (w/replicates)	Sampling SOP	Sample Volume	Containers #, size, type	Preservation (chemical, temperature, light protected)	Maximum Holding Time: Preparation/ Analysis
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Conductivity	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Dissolved Oxygen	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	pH	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Temperature	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Turbidity	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Flow	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Ammonia	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Chlorine	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Copper	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Surfactants	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Canine Scent Tracking	QAPP Sections 10 & 11 or MP	Section 11.2	Instream	NA	NA	NA: Site measurement

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Table 11-2. Sample collection for constituents for laboratory analysis (also see discussion in QAPP Section 10 or Monitoring Plan)

Sampling Location	Site ID Number	Matrix	Depth (units)	Analytical Parameter	No. Samples (w/replicates)	Sampling SOP	Sample Volume	Containers #, size, type	Preservation (chemical, temperature, light protected)	Maximum Holding Time: Preparation/ Analysis
Laboratory Analyses										
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	<i>E. coli</i>	QAPP Sections 10 & 11 or MP	Section 11.1.1	100 or 125 mL	1 bottle, 125 mL, sterile plastic (high density polyethylene or polypropylene)	Sodium thiosulfate pre-added to containers in the laboratory (chlorine elimination). Cool to 4 °C; dark	6 hours at 4 °C, dark; laboratory must be notified well in advance
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	<i>Enterococcus</i>	QAPP Sections 10 & 11 or MP	Section 11.1.1	100 or 125 mL	1 bottle, 125 mL, sterile plastic (high density polyethylene or polypropylene)	Sodium thiosulfate pre-added to containers in the laboratory (chlorine elimination). Cool to 4 °C; dark	6 hours at 4 °C, dark; laboratory must be notified well in advance
QAPP Sections 6 & 10 or MP	See MP	Sediment	Sediment surface	<i>E. coli</i>	QAPP Sections 10 & 11 or MP	Section 11.1.2	125 mL	10 grams	1 50 mL sterile conical tube	Cool to 4 °C, dark
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	TSS	QAPP Sections 10 & 11 or MP	Section 11.1.1	1000 mL	1 bottle, 1000 mL, cool to 4 °C, dark	Cool to 4 °C, dark	7 days at 4 °C, dark
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Ammonia	QAPP Sections 10 & 11 or MP	Section 11.4.2	100 mL	1 bottle, 100 mL, cool to 4 °C, high density polyethylene, dark	Sulfuric acid pre-added to containers in the laboratory. Cool to 4 °C, dark	28 days at 4 °C, dark
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Potassium	QAPP Sections 10 & 11 or MP	Section 11.4.2	500 mL	1 bottle, 100 mL, cool to 4 °C, high density polyethylene or glass, dark	Nitric acid pre-added to containers in the laboratory. Cool to 4 °C, dark	6 months at 4 °C, dark
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Surfactants	QAPP Sections 10 & 11 or MP	Section 11.4.2	500 mL	1 bottle, 100 mL, cool to 4 °C, high density polyethylene or glass, dark	Cool to 4 °C, dark	48 hours at 4 °C, dark
Molecular Analyses										
QAPP Sections 6 & 10 or MP	See MP	Water	Water surface	Genetic markers for human and canine (<i>Bacteroides thetaiotaomicron</i>), horse (<i>Bacteroides spp.</i>), bird (<i>Catelliboccus</i>), and rumen (<i>Prevotella</i>)	QAPP Sections 10 & 11 or MP	Section 11.1.1	1000 mL	1 bottle, 1000 mL, cool to 4 °C; dark	Cool to 4 °C; dark	24 hours at 4 °C, dark; laboratory must be notified in advance
QAPP Sections 6 & 10 or MP	See MP	Sediment	Sediment surface	Genetic markers for human and canine (<i>Bacteroides thetaiotaomicron</i>), horse (<i>Bacteroides spp.</i>), bird (<i>Catelliboccus</i>), and rumen (<i>Prevotella</i>)	QAPP Sections 10 & 11 or MP	Section 11.1.1	10 grams	1 50 mL sterile conical tube	Cool to 4 °C; dark	24 hours at 4 °C, dark; laboratory must be notified in advance

- (1) Stretch the measuring tape across the stream at right angles to the direction of flow. When using an electronic flow meter, the tape does not have to be exactly perpendicular to the bank (direction of flow). Avoid measuring flow in areas with back eddies. The first choice would be to select a site with no back eddy development. However, this cannot be avoided in certain situations. Measure the negative flows in the areas with back eddies. If necessary and possible, modify the measuring cross section to provide acceptable conditions by building dikes to cut off dead water and shallow flows, remove rocks, weeds, and debris in the reach of stream one or two meters upstream from the measurement cross section. After modifying a streambed, allow the flow to stabilize before starting the flow measurement
- (2) Record the following information on the flow measurement form (Attachment 3):
 - (a) Monitoring site and Site ID
 - (b) Date
 - (c) Time measurement is initiated and ended
 - (d) Name of person(s) measuring flow
 - (e) Note if measurements are in feet or meters
 - (f) Total stream width and width of each measurement section
 - (g) For each cross-section, record the mid-point, section depth, and flow velocity
- (3) Determine the spacing and location of flow measurement sections. Measurements will be taken at the midpoint of each of the flow measurement sections. Flow measurements will be taken at the following locations:
 - (a) A point from the left bank representing 10 percent of the total width. This measurement will provide a velocity estimate for the section representing 0 percent – 20 percent of the total width from the left bank.
 - (b) A point from the left bank representing 50 percent of the total width. This measurement will provide a velocity estimate for the section representing 20 percent – 80 percent of the total width from the left bank.
 - (c) A point from the left bank representing 90 percent of the total width. This measurement will provide a velocity estimate for the section representing 80 percent – 100 percent of the total width from the left bank.
- (4) Place the top setting wading rod at each flow measurement point.
- (5) Using a tape measure, measure the depth of water to the nearest ½ inch.

- (6) Adjust the position of the sensor to the correct depth at each flow measurement point. The purpose of the top setting wading rod is to allow the user to easily set the sensor at 20 percent, 60 percent, and 80 percent of the total depth. On the wading rod, each single mark represents 0.10 foot, each double mark represents 0.50 foot, and each triple mark represents 1.00 foot. Position the meter at 60 percent of the total depth from the water surface (if depth of flow is greater than 2.5ft, then take two readings, at 20 percent and 80 percent of total depth).
- (7) Measure and record the velocity and depth. The wading rod is kept vertical and the flow sensor kept perpendicular to the cross section. Permit the meter to adjust to the current for a few seconds. Measure the velocity for a minimum of 20 seconds with the Marsh-McBirney meter. When measuring the flow by wading, stand in the position that least affects the velocity of the water passing the current meter. The person wading stands a minimum of 1.5 feet downstream and off to the side of the flow sensor.
- (8) Report flow values less than 10 ft³/s to two significant figures. Report flow values greater than 10 ft³/s to the nearest whole number, but no more than three significant figures.
- (9) Calculate flow by multiplying the width x depth (ft²) to derive the area of each flow measurement section. The area of the section is then multiplied by the velocity (ft/s) to calculate the flow in cubic feet per second (cfs or ft³/sec) for each flow measurement section. Do not treat cross sections with negative flow values as zero. Negative values obtained from areas with back eddies should be subtracted during the summation of the flow for a site. When flow is calculated for all of the measurement sections, they are added together for the total stream flow.

11.4 Secondary Screening Tools

The following is a summary of secondary screening tools that can be used while conducting Tier 2 source evaluation activities.

11.4.1 Storm Drain Visual Observations (including flow)

Determination of flow within a storm drain during dry weather can provide an understanding of the magnitude of a potentially illicit discharge during dry weather.

11.4.1.1 Manhole Cover Removal Procedures

Underground MS4 systems may require the removal of manholes to assess the presence of dry weather flow. The process for removing the manhole cover is based on the process described as follows (Center for Watershed Protection, *Illicit Discharge Detection and Elimination Guide* (2004) :

- (1) Locate the manhole cover to be removed.
- (2) Divert road and foot traffic away from the manhole using traffic cones. For more information on traffic control, see the California Manual on Uniform Traffic Control Devices guideline for temporary traffic control (2006).
- (3) Use the tip of a crowbar to lift the manhole cover up high enough to insert the gas monitor probe. Take care to avoid creating a spark that could ignite explosive gases that may have accumulated under the lid.

- (4) Follow procedures outlined for the gas monitor to test for accumulated gases.
- (5) If the gas monitor alarm sounds, close the manhole immediately. Do not attempt to open the manhole until sometime is allowed for gases to dissipate.
- (6) If the gas monitor indicates the area is clear of hazards, remove the monitor probe and position the manhole hook under the flange. Remove the crowbar. Pull the lid off with the hook.
- (7) When testing is completed and the manhole is no longer needed, use the manhole hook to pull the cover back in place. Make sure the lid is settled in the flange securely.
- (8) Check the area to ensure that all equipment is removed from the area prior to leaving.

The following safety considerations should be taken into account when sampling from a manhole:

- (1) Do not lift the manhole cover with your back muscles.
- (2) Wear steel-toed boots or safety shoes to protect feet from possible crushing injuries that could occur while handling manhole covers.
- (3) Do not move manhole covers with hands or fingers.
- (4) Wear safety vests or reflective clothing so that the field crew will be visible to traffic.
- (5) Manholes may only be entered by properly trained and equipped personnel and when all OSHA and local rules are followed.

References

California Department of Transportation (Caltrans). 2006. *California Manual on Uniform Traffic Control Devices for Streets and Highways, Part 6: Temporary Traffic Control*. Available online at:
<http://www.dot.ca.gov/hq/traffops/signtech/mutcdsupp/pdf/camutcd/CAMUTCD-Part6.pdf>

11.4.1.2 Storm Drain Visual Observations

Next, visually inspect inside the storm drain for the presence or absence of dry weather flow. If flow is present, other observations regarding the storm drain discharge may include presence of staining, odors, floatable materials, or colors. Record observations on field data sheet or log book.

11.4.1.3 Estimate Depth of Water in Storm Drain

If there appears to be a significant amount of flow, additional observations may be desired regarding the amount of water that is present within the storm drain. This procedure is loosely based on Oklahoma State Extension Service (2000) and US EPA (1989).

- (1) Remove manhole as described in Section 11.4.1.1.
- (2) Prepare steel measuring tape with lead weight at end or telescoping survey rod for use by running carpenter chalk along the last few feet of the tape or survey rod.

- (3) Place the steel tape or survey rod into the manhole and ensure that they are completely submerged, reaching the bottom of the manhole. Care should be taken to ensure the steel tap or rod stay perpendicular to the bottom of the manhole and that the steel tape does not bend.
- (4) Pull the tape or rod back up to ground surface and observe the point at which a color change between dry and wet chalk occurs. This line denotes the length of tape/rod that was immersed in water.
- (5) Record the depth measurement on field sheet or log book.

References

Center for Watershed Protection. 2004. *Illicit Discharge Detection and Elimination: A Guidance Manual for Program Development and Technical Assessments*. Available online at:

http://cfpub.epa.gov/npdes/docs.cfm?program_id=6&view=allprog&sort=name#iddemmanual

Oregon State Extension Service. 2000. *Measuring Water Well Levels*. Available online at:

<http://extension.oregonstate.edu/catalog/pdf/ec/ec1368.pdf>

US EPA. 1989. *Accuracy of Depth to Water Measurements*. Available online at:

<http://www.epa.gov/superfund/remedytech/tsp/download/accur.pdf>

11.4.2 Field-based Monitoring Procedures

There are several useful monitoring procedures that can be used to conduct secondary screening to support bacteria source evaluation activities. The following section summarizes a menu of options that can be applied when evaluating potential sources of bacteria from an outfall or storm drain exhibiting dry weather flow.

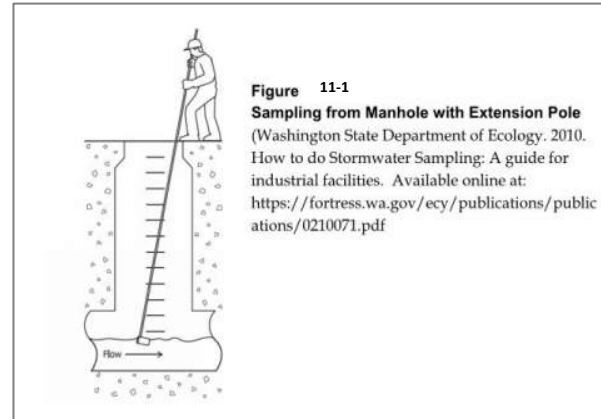
11.4.2.1 Sample collection

Underground storm sewer sampling may be accomplished without entering the manhole by utilizing an intermediate sampling device, such as an extension pole with a sampling bottle/bag (Figure 11-1).

Procedures adapted from Washington State Department of Ecology (2010).

- (1) Proceed to sample location. Remove manhole cover as described in Section 11.4.1.1.
- (2) Appropriate gloves (latex or rubber) are worn **at all times** when handling samples or conducting test kit analyses. Other appropriate Personal Protection Equipment (PPE) should be worn, as required.
- (3) Secure clean sample bottle or bag to sampling pole. This may necessitate the use of filament strapping tape or zip ties.
- (4) Lower sampling devise into sewer. Holding the device so the bottle or bag faces upstream, allow the water to enter the sampler. Rinse the sample bottle or bag at least three times with flowing sample water.

- (5) Take sample from the central portion of flow in an area of some turbulence, if possible.
- (6) Raise sampling device out of manhole and, keeping hands from the opening of the bottle or bag, fill sample containers. Repeat steps 4-6 as necessary until all sample containers are filled.
- (7) Repeat steps 4-6 as necessary to measure field parameters.
- (8) Note on field sheets whether flow is coming from laterals or main sewer pipe. If coming from laterals, note the direction of flow (e.g., NW lateral).



References

Washington State Department of Ecology. 2010. *How to do Stormwater Sampling: A Guide for Industrial Facilities*. Available online at: <https://fortress.wa.gov/ecy/publications/publications/0210071.pdf>

11.4.2.2 Ammonia Test Strips

Nitrogen is a fundamental nutrient in the aquatic ecosystem and is required for survival by all plants and animals. In aquatic ecosystems, nitrogen is present in different forms: nitrate, nitrite, ammonia, and organic nitrogen. Of particular interest to storm drain systems is ammonia-nitrogen, which could indicate illegal wastewater connections to the sanitary sewer system, poorly functioning septic systems, or wildlife.

Implementation of the following procedures will require that the field personnel be equipped with ammonia test strips by Hach or similar manufacturer.

- (1) To use the ammonia test strips, gloves should first be donned. Appropriate gloves (latex or rubber) are worn **at all times** when handling samples or conducting test kit analyses. Other appropriate PPE should be worn, as required.
- (2) A sample should then be collected from an outfall or storm sewer line using a sample dipper or other sample collection tool as described in Section 11.4.2.1.
- (3) Samples for ammonia will be poured directly from the sample collection tool into a sample cup which will be rinsed three times with the sample.
- (4) Analysis will proceed as directed on the ammonia test strip box but will generally proceed in the following manner:
 - (a) Remove one test strip from the box. Replace top of box tightly immediately.
 - (b) Dip the test strip into the water sample for the suggest time (5 to 30 seconds). The time the strip is submerged will depend on the brand of test strip. Vigorously move the strip up and down in the water, making sure the test strip pad is always submerged.

- (c) Remove the test strip from the water and shake off any excess water. Wait the suggested amount of time for the test strip to change color.
 - (d) To read result, turn test strip over so that the testing pad is facing away from you.
 - (e) Compare the color of the test strip pad to the color chart above. Estimate results if the color on the test strip falls between two color blocks.
- (5) The results of the analysis will be recorded on a field sheet or log book.
- (6) The sample in the cup can be discarded and the sample cup should be rinsed twice with deionized water.

11.4.2.3 Chlorine Test Strips

Chlorine is used in water treatment and wastewater treatment processes to disinfect water. Presence of chlorine in storm drain discharges could indicate an illicit connection with the water supply system, wastewater effluent or another human source.

There are different types of chlorine analyses available for use in the field. Test strips are available from Hach for chlorine residual (i.e., free chlorine); test kits are also available using the N,N-Diethylparaphenylenediamine (DPD) method which will cause a color change which can then be evaluated using color discs or field spectrophotometers.

Procedures are provided in the following section for chlorine residual test strips. Other analyses should proceed as directed in test equipment SOPs.

- (1) To use the chlorine test strips, gloves should first be donned. Appropriate gloves (latex or rubber) are worn at **all times** when handling samples or conducting test kit analyses. Other appropriate PPE should be worn, as required.
- (2) A sample should be collected from an outfall or storm sewer line using a sample dipper or other sample collection tool as described in Section 11.4.2.1.
- (3) Samples for chlorine will be poured directly from the sample collection tool into a sample cup which will be rinsed three times with the sample.
- (4) Analysis will proceed as directed on the chlorine test strip box but will generally proceed in the following manner:
 - (a) Remove one test strip from the box. Replace top of box tightly immediately.
 - (b) Dip the test strip into the water sample for the suggest time (5 to 30 seconds). The time the strip is submerged will depend on the brand of test strip. Vigorously move the strip up and down in the water, making sure the test strip pad is always submerged.
 - (c) Remove the test strip from the water and shake off any excess water. Wait the suggested amount of time for the test strip to change color.

- (d) To read result, turn test strip over so that the testing pad is facing away from you.
 - (e) Compare the color of the test strip pad to the color chart above. Estimate results if the color on the test strip falls between two color blocks.
- (5) The results of the analysis will be recorded on a field sheet or log book.
- (6) The sample in the cup can be discarded and the sample cup should be rinsed twice with deionized water.

11.4.2.4 Copper Test Strips

Copper is a metallic element essential to human growth and is literally found all over the world. Detection of copper during secondary screening may indicate an illicit discharge into the storm drain system from human sources, such as algicides, copper pipes, or electrical components.

There are different types of copper field analyses available for use. Test strips are available from Hach for copper providing readings between 0 and 3 mg/L while colorimetric test kits are also available and provide more precise readings between 0.2 and 5 mg/L.

Procedures are provided in the following section for copper test strips. Other analyses should proceed as directed in test equipment SOPs.

- (1) To use the copper test strips, gloves should first be donned. Appropriate gloves (latex or rubber) are worn **at all times** when handling samples or conducting test kit analyses. Other appropriate PPE should be worn, as required.
- (2) A sample should be collected from an outfall or storm sewer line using a sample dipper or other sample collection tool as described in Section 11.4.2.1.
- (3) Samples for copper will be poured directly from the sample collection tool into a sample cup which will be rinsed three times with the sample.
- (4) Analysis will proceed as directed on the copper test strip box but will generally proceed in the following manner:
 - (a) Remove one test strip from the box. Replace top of box tightly immediately.
 - (b) Dip the test strip into the water sample for the suggest time (5 to 30 seconds). The time the strip is submerged will depend on the brand of test strip. Vigorously move the strip up and down in the water, making sure the test strip pad is always submerged.
 - (c) Remove the test strip from the water and shake off any excess water. Wait the suggested amount of time for the test strip to change color.
 - (d) To read result, turn test strip over so that the testing pad is facing away from you.

- (e) Compare the color of the test strip pad to the color chart above. Estimate results if the color on the test strip falls between two color blocks.
- (5) The results of the analysis will be recorded on a field sheet or log book.
- (6) The sample in the cup can be discarded and the sample cup should be rinsed twice with deionized water.

11.4.2.5 Surfactant/Detergent Colorimetric Screening

Many illicit discharges into storm drains will have elevated concentrations of surfactants and detergents. Industrial cleaning, commercial wash water and car washes may also be sources of surfactants and detergents in storm drains. Leaking sanitary sewers could also contribute detergents used in household cleaning.

Procedures are provided in the following section for the Hach Detergents Test Kit (Model DE-2). Other analyses should proceed as directed in test equipment SOPs.

- (1) To use the detergent test kit, gloves should first be donned. Appropriate gloves (latex or rubber) are worn **at all times** when handling samples or conducting test kit analyses. Other appropriate PPE should be worn, as required.
- (2) Prepare a sample from the outfall/storm drain dry weather discharge
 - (a) A sample should be collected from an outfall or storm sewer line using a sample dipper or other sample collection tool as described in Section 11.4.2.1.
 - (b) Rinse the test tube three times with sample water.
 - (c) Pour 20 mL directly from the sample collection tool directly into the provided test tube (20 mL will be the upper mark on the test tube).
 - (d) Add 12 drops of the Detergent Test Solution. Place stopper on test tube and shake to mix.
 - (e) Add chloroform to the lowest mark (5 mL) on the test tube. Chloroform is heavier than water and will sink. Place stopper on test tube and vigorously shake for 30 seconds. All test tube to stand for 1 minute to allow chloroform to separate.
 - (f) Using the draw off pipet provided in the test kit, remove water from the test tube and discard.
 - (g) Refill the test tube to the upper 20 mL mark with the Wash Water buffer. Then immediately use the draw off pipet to remove the Wash Water buffer and discard. This step washes away the remaining water sample.
 - (h) Should the sample be turbid, it may be necessary to filter the chloroform solution. If this is the case, the following steps should be followed:
 - (i) Place a small ball (about the size of a large pea) of glass wool in the filter thimble.

- (ii) Using the draw off pipet, remove the chloroform and filter through the glass wool back into the test tube
- (i) Refill the test tube to the upper mark with the Wash Water buffer, place stopper on the test tube and shake vigorously for 30 seconds. Allow to stand one minute to allow chloroform to separate.
- (3) While waiting for the chloroform to separate, fill another test tube with demineralized water and place it in the left opening of the color comparator.
- (4) Insert the test tube containing the prepared sample into the right opening of the color comparator.
- (5) Hold the comparator up to a light and view through the two openings in the front. Rotate the Detergents Color Disc until a color match is obtained. Read the ppm Detergents from the scale window.
- (6) The results of the analysis will be recorded on a field sheet or log book.
- (7) The sample in the cup can be discarded into a container. The sample cup should be rinsed twice with deionized water and also poured into container for disposal at a later time.

If the color is darker than the highest reading on the color disc, a sample dilution can be performed. To prepare a 20:1 dilution, add 1 mL sample and filling test tube with demineralized water to the 20 mL mark. Follow sample preparation process outlined in Step 2 of this procedure and re-analyze the sample.

It should also be noted that this test may generate waste that is considered hazardous. This waste cannot be dumped into the sanitary sewer system but must be collected and disposed of properly

References

Center for Watershed Protection. 2004. *Illicit Discharge Detection and Elimination: A Guidance Manual for Program Development and Technical Assessments*. Available online at:
http://cfpub.epa.gov/npdes/docs.cfm?program_id=6&view=allprog&sort=name#iddmanual

11.4.2.6 Canine Scent tracking

The use of canines to track human sources of storm drain illicit discharges have been reported as an accurate method that results in very few false positives (Murray et al., 2011). Canine scent tracking should be used to assist in locating specific sources of human-specific bacteria within a storm drain system as follows.

- (1) A provider of canine scent tracking should be contacted to secure a dog-handler pair to conduct the monitoring. One provider of canine scent tracking is Environmental Canine Services, LLC.
- (2) If operating canine scent tracking in the field, proceed to storm drain of interest with dog-handler pair. If operating canine scent tracking in the laboratory or office, skip to next section.
- (3) At a storm drain of interest, remove the manhole cover as described in Section 11.4.1.1

- (4) Once the cover is removed, the handler will give the canine its individual search command and walk to the open structure.
- (5) If the canine alerts at the storm drain, the handler will provide interpretation to confirm the presence or absence of human sewage in the storm drain.
- (6) The results of the canine scent tracking should be recorded on a field data sheet or logbook.

Canine scent tracking may also be used in a laboratory or office setting as follows. Methods are adapted from the Ottawa County Health Department (2011).

- (1) Contact a provider of canine scent tracking to secure a dog-handler pair. Prior to sampling, coordinate a time when sampling will be complete to meet dog-handler pair in a scent-neutral area.
- (2) Proceed to storm drain of interest with sampling team only.
- (3) At a storm drain of interest, remove the manhole cover as described in Section 11.4.1.1
- (4) Once the cover is removed, proceed to take a sample according to procedures outlined in Section 11.4.2.1. Collect at least one 60 mL sample.
- (5) Preserve sample on ice at 4 °C. Store for no longer than 8 hours.
- (6) Proceed to scent-neutral area to conduct canine scent sampling. Canine scent sampling must be completed within 8 hours of sample collection.
- (7) If canine alerts at the sample, the handler will provide interpretation to confirm the presence or absence of human sewage in the sample.
- (8) The results of the canine scent tracking should be recorded on a field sheet or logbook.

References

Murray, Jill, Scott Reynolds, Patricia Holden, Laurie Van De Werfhorst. 2011. *Canine Scent and Microbial Source Tracking in Santa Barbara, California*. Water Environment Research Foundation Report U2R09.

Ottawa County Health Department. 2011. *Ottawa County Health Department's Beach Monitoring Project Quality Assurance Project Plan (QAPP)*.

12. Sample Handling and Custody

12.1 Pre-Sampling Procedures

Prior to the collection of field data, the sample team will complete the following activities:

- (1) Prepare and calibrate a multi-parameter instrument for use in collecting field measurements prior to sampling (See the equipment operation manual for specific calibration instructions). Calibrations will be conducted by the Responsible Agency's Project QA Officer or Monitoring Manager or their designee. Sampling activities will not be conducted until calibrations can be completed per equipment operations manual.
- (2) Gather equipment for measurement of field parameters, including multi-parameter instrument, applicable test strips and test kits, and, if sampling underground storm drains, sampling pole.
- (3) Prepare and calibrate a portable Turbidity Meter (e.g., Hach or equivalent), as necessary.
- (4) Prepare ice coolers with ice packs or crushed ice to transport samples to the laboratory.
- (5) Obtain sample containers from laboratories, including bottles for field blanks and water collection bottles. For sampling underground storm drains, also obtain sterile whirl-pak® bags or equivalent, if necessary.
- (6) Prepare pre-label sampling containers as appropriate, e.g., Site ID, Sample ID, and Project ID, and leave blank fields for date and time.
- (7) Prepare a solution of 70 percent ethanol for field sterilization of sampling equipment.
- (8) Pack a flat head screw driver – used to loosen the band that holds the sampling bottle to the sampling pole.
- (9) Check safety gear, including rubber boots and waders, protective gloves, and safety vests.
- (10) Pack a waterproof pen and field log book and/or field data sheets.
- (11) Pack peristaltic pump and sterile tubing.
- (12) Pack box cutter and razor blades.
- (13) Pack duct tape.
- (14) Prepare vehicle, including fueling.
- (15) Pack supplies for shipping samples, if applicable.
- (16) Pack chain of custody forms, field data sheets, camera with flash, and zip lock bags.
- (17) Ensure keys to monitoring sites with locked access are available.

12.2 Field Documentation

Field crews are required to keep a field log or complete appropriate data forms. Field documentation will be completed using indelible ink, with any corrections made by drawing a single line through the error and entering the correct value. Electronic mobile databases may be used in place of a field log or data forms to directly input data from the field. Taking into account the type of sampling being conducted, e.g., Regional Monitoring Program vs. TMDL Program monitoring, the following items should be recorded in the field log or on data forms for each sample collected at each monitoring site (An example Field Data Sheet Form is included as Attachment 1):

- Date and time of sample collection.
- Site Name and Site ID.
- Unique identification numbers for any replicate or blank samples collected from the site.
- Site IDs of the proximate upstream and downstream sampling locations (for Tier 2 urban source evaluation screening investigations only).
- The results of any field measurements (conductivity, dissolved oxygen, flow, pH, temperature, turbidity, ammonia, chlorine, copper, and detergents) and the time that measurements were made. For underground storm drain sampling, depth measurements may be reported in place of flow.
- Qualitative descriptions of relevant water conditions (e.g., color, flow level, clarity, or odor) or weather (e.g., wind, rain) at the time of sample collection.
- For collection of samples to evaluate bacteria sources, a qualitative description of the surrounding drainage area including evidence of flow in street gutters, presence of road sediments and debris, and indications of excess irrigation. Also note the approximate surface area draining to the inlet.
- For bacteria source evaluation sites, when such characterizations are required, a characterization of the hydrologic connectivity of the surface flow at the site to the downstream impaired water to which it is tributary. If no connectivity is observed, then the characterization shall, at a minimum, describe the general distance between the point where surface flow ceases and the channel confluences with the downstream impaired water. If connectivity is observed, then the characterization shall, at a minimum, describe the typical width and depth of the surface flow reaching the downstream impaired water, any observations that suggest that flows have recently been higher than what is currently observed.
- A description of any unusual occurrences associated with the sampling of that site, particularly those that may affect sample or data quality.

Field crews are required to take digital photographs when sampling each site and maintain a photo log of all photographs taken. At a minimum, the following digital photographs should be taken at each site, regardless of the purpose for sampling:

- A photograph which shows a view of the waterbody upstream of the sample site;
- A photograph which shows a view of the waterbody downstream of the sample site; and

- Photographs which characterize the width and depth of flow and aesthetic characteristics such as water clarity and algal growth.

For Tier 2 urban source evaluations, the following photographs should be taken:

- A photograph which shows the drain inlet;
- A photograph which shows the sampling point inside the storm sewer (it may be necessary to utilize a camera with flash enabled);
- A photograph which shows the drainage area upstream of the sample site; and
- A photograph which shows the drainage area downstream of the sample site

To the extent possible, the photographs that provide an upstream and downstream view of the waterbody should be taken from the same point during each site visit. A photo log of all photographs taken at each sample site shall be maintained that documents the purpose of each photograph (for example, upstream or downstream view) and the date and time of the photograph.

12.3 Sampling Handling, Delivery to Laboratory and Chain of Custody

Proper gloves must be worn to prevent contamination of the sample and to protect the sampler from environmental hazards (disposable polyethylene, nitrile, or non-talc latex gloves are recommended). Wear at least one layer of gloves, but two layers help protect against leaks. One layer of shoulder high gloves worn as first (inside) layer is recommended to have the best protection for the sampler. Safety precautions are needed when collecting samples, especially samples that are suspected to contain hazardous substances, bacteria, or viruses.

Properly store and preserve samples as soon as possible. Usually this is done immediately after returning from the collection by placing the containers on top of bagged, crushed or cube ice in an ice chest. Sufficient ice will be needed to lower the sample temperature to at least 4 °C within 45 minutes after time of collection. Sample temperature will be maintained at 4 °C until delivered to the appropriate laboratory. Care should be taken at all times during sample collection, handling, and transport to prevent exposure of the sample to direct sunlight.

Samples that are to be analyzed for bacterial indicators must be kept on ice or in a refrigerator and delivered to a qualified laboratory included in the appendices of this QAPP within 6 hours of sample collection.

Samples analyzed for *Bacteroides* must be kept on ice or in a refrigerator and delivered to a qualified laboratory included in the appendices of this QAPP within 24 hours of collection.

A detailed sample delivery schedule is presented in Tables 10-1 and 10-2 of this QAPP for collection of water samples from RMP Priority 1, 2, and 3 sites. Other monitoring programs have flexible schedules.

Samples will be delivered to analytical laboratories by the Responsible Agency's sampling personnel either directly or via courier.

Every shipment must contain a complete COC Form (see Attachment 2) that lists all samples taken and the analyses to be performed on these samples. COCs must be completed every time samples are transported to a laboratory. Include any special instructions to the laboratory. The original COC sheet (not the copies) is included with the shipment (insert into zip lock bag); one copy goes to the sampling coordinator; and the sampling crew keeps one copy. Samples collected should have the depth of collection and date/time collected on every COC.

Due to increased shipping restrictions, samples being sent via a freight carrier require additional packing. Although care is taken in sealing the ice chest, leaks can and do occur. Samples and ice should be placed inside a large plastic bag inside the ice chest for shipping. The bag can be sealed by simply twisting the bag closed (while removing excess air) and taping the tail down. Prior to shipping the drain plug of the ice chests have to be taped shut. Leaking ice chests can cause samples to be returned or arrive at the laboratory beyond the required holding time. Although glass containers are acceptable for sample collection, bubble wrap must be used when shipping glass.

13. Analytical Methods

Samples collected for the Regional and TMDL Monitoring Programs will be analyzed for various chemical and biological constituents. Field parameters will be monitored at the sampling sites using a multi-parameter water quality probe (or equivalent) and includes conductivity, dissolved oxygen, pH, temperature, and turbidity. Additional constituents (ammonia, chlorine, copper, and surfactants) will be quantified in the field using Hach Company water chemistry kits. Samples for biological constituents, *E.coli*, *Enterococcus* and *Bacteroides*, and other chemical constituents will be quantified at a qualified laboratory.

Multiple EPA approved methods may be used to analyze *E.coli* or *Enterococcus* concentrations in water samples including (a) EPA Method 1603, Standard Methods (SM) 9223B, and IDEXX (18 hour) for *E coli*; and (b) EPA Method 1600 and IDEXX Enterolert for *Enterococcus*. Sediment samples will be sonicated to release all *E.coli* from sediment and biofilms and then analyzed using method EPA 1603.

Analytical methods used to quantify constituent levels are summarized in Tables 13-1 and 13-2.

Table 13-1. Analytical methods for field parameters

Analyte	Laboratory / Organization	Project Action Limit (units, wet or dry weight)	Target Reporting Limit (units, wet or dry weight)	Field Method	
				Analytical Method/ SOP ²	Modified for Method (Yes/No)
Conductivity ¹	Field monitoring	1.09 µS/cm	0 - 100 µS/cm	SM 2510B	No
Dissolved Oxygen	Field monitoring	5 mg/L	0 - 19.9 mg/L	SM 4500OG	No
pH	Field monitoring	6.5 to 8.5	0 – 14 pH Units	SM 4500-H+B	No
Temperature (water) ³	Field monitoring	June to Oct: not > 90 °F (32°C); Rest of Year: not > 78°F (25°C) as a result of controllable water quality factors	0 – 50 °C	SM 2550B	No
Turbidity	Field monitoring	5 to 10 Nephelometric Units (NTU)	0 – 800 NTU	SM 2130B	No
Flow	Field monitoring	NA	-0.5 to 19.99 ft/sec	Cross-section velocity profile or Visual flow estimate (see text)	No
Ammonia ⁴	Field monitoring	1 mg/L	0 – 6 mg/L ⁵	NA	No
Chlorine ⁴	Field monitoring	NA	0 – 10 mg/L ⁵	NA	No
Copper ⁴	Field monitoring	0.1 mg/L	0 – 3 mg/L ⁵	NA	No
Surfactants ⁴	Field monitoring	0.01 mg/L	0 – 3 mg/L ⁶	NA	No
Canine Scent Tracking ⁴	Field monitoring	Positive detection indicated by vocalization or active response	No positive response -- Positive response	NA	No

Notes:

¹ Project Action Limits: Applied Basin Plan Water Quality Objectives for conductivity by converting a total dissolved solids value of 700 ppm to a conductivity value.

² SM: *Standard Methods for the Examination of Water and Wastewater*, 20th edition.

³ Urban Source Evaluation Monitoring Program will only measure water temperature

⁴ Optional Tier 2 secondary screening methodologies; Project Action Limits based on potential ranges for chemical tracers indicating sewage, as indicated in monitoring plan.

⁵ Target Reporting Limit based on test kits sold and distributed by Hach; other manufacturers may specify alternative reporting limits.

⁶ Target Reporting Limit based on test kit sold and distributed by Chemets; other manufacturers may specify alternative reporting limits.

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Table 13-2. Laboratory analytical methods

Analyte	Laboratory/ Organization	Project Action Limit (units, wet or dry weight)	Target Reporting Limit (units, wet or dry weight)	Analytical Method		Achievable Laboratory Limits	
				Analytical Method/ SOP	Modified for Method (Yes/No)	Method Detection Limits	Method
<i>E. coli</i>	OC Public Health Water Quality Lab	See notes below ¹	10 cfu/100 mL	EPA 1603 ²	No	Not applicable	Varies ³
<i>E. coli</i>	OC Public Health Water Quality Lab; Babcock Laboratories; Clinical Lab	See notes below ¹	10 Most Probable Number (MPN)/ 100 mL	SM 9223B IDEXX 18HR	No	Not applicable	Varies ³
<i>Enterococcus</i>	OC Public Health Water Quality Lab	See notes below ¹	10 cfu/100 mL	EPA 1600	No	Not applicable	Varies ³
<i>Enterococcus</i>	OC Public Health Water Quality Lab	See notes below ¹	10 cfu/100 mL	IDEXX Enterolert	No	Not applicable	Varies ³
Genetic markers for human and canine (<i>Bacteroides</i> <i>thetaiotaomicron</i> ⁴), bird (<i>Catelliboccus</i>), and rumen (<i>Prevotella</i>)	Orange County Water District	Presence / Absence	10 gene copies / 1000 mL	qPCR assays	No	Not applicable	10 gene copies/ 1000 mL
Genetic markers for horse (<i>Bacteroides</i>)	Weston Solutions	Presence / Absence	10 gene copies / 1000 mL	qPCR HoF597 assay	No	Not applicable	10 gene copies / 1000 mL
Total Suspended Solids	OC Public Health Water Quality Lab; Associated Labs; Weck Labs; Babcock Laboratories; Clinical Lab	See notes below ⁵	1.0 mg/L	SM 2540D	No	Not applicable	1.0 mg/L
Ammonia	Babcock Laboratories	1.0 mg/L; Ammonia/ Potassium Ratio > 0.6 mg/L	0.1 mg/L	SM 4500	No	Not applicable	0.1 mg/L

Table 13-2. Laboratory analytical methods

Analyte	Laboratory/ Organization	Project Action Limit (units, wet or dry weight)	Target Reporting Limit (units, wet or dry weight)	Analytical Method		Achievable Laboratory Limits	
				Analytical Method/ SOP	Modified for Method (Yes/No)	Method Detection Limits	Method
Ammonia	Clinical Lab	1.0 mg/L; Ammonia/ Potassium Ratio > 0.6 mg/L	0.1 mg/L	EPA 350.1	No	Not applicable	0.01 mg/L
Potassium	Babcock Laboratories; Clinical Lab	Ammonia/ Potassium Ratio > 0.6 mg/L	NA	EPA 200.7	No	Not applicable	1.0 mg/L
Surfactants (MBAS)	Babcock Laboratories	0.01 mg/L	NA	SM 5540C	No	Not applicable	0.05 mg/L
Surfactants (MBAS)	Clinical Lab	0.01 mg/L	NA	SM 5540C	No	Not applicable	0.1 mg/L

Notes:

¹ Project Action Limits for *E. coli* and TSS are as follows: (based on the TMDL): *E. coli*: 5-sample/30-day Logarithmic Mean less than 113 organisms/100 mL, and not more than 10% of the samples exceed 212 organisms/100 mL for any 30-day period.

² Sediment samples will be sonicated to release all *E. coli* from sediment and biofilms and then analyzed using method EPA 1603.

³ The achievable laboratory limits are dependent on analytical methods and sample dilutions conducted by laboratories.

⁴ Analytical reference for *Bacteroides thetaiotaomicron*: Sauer E.P., J.L. VandeWalle, M.J. Bootsma, and S.L. McLellan. 2011. *Detection of Human Specific Bacteroides Genetic Marker Provides Evidence of Widespread Sewage Contamination of Stormwater in the Urban Environment*. Water Research 45 (2011) 4081-4091.

⁵ TSS: in inland surface waters shall not contain suspended or settleable solids in amounts which cause a nuisance or adversely affect beneficial uses as a result of controllable water quality factors.

14. Quality Control

All contract laboratories used to implement the SAR Bacteria Monitoring Plan will follow QA/QC programs in accordance with guidelines established by the State of California and the U.S. EPA. Laboratories are required to submit a copy of their SOPs for laboratory quality control to the Responsible Agency's Project QA Officer for review and approval (see Appendices to this QAPP for the SOPs of laboratories being used by this project).

All field and laboratory data will be entered by the Responsible Agency's Data Manager into a database/spreadsheet template provided by the Project Director. Annually, after the end of a sample year, each Responsible Agency's Project Manager will submit the previous sample year's completed database/spreadsheet to the Project Director to support preparation of the Annual Report. The Project Director will upload all previous sample year data to CEDEN. Any electronic or paper files will be filed in the project archives maintained by the Project Director along with related materials such as field forms, chain of custody forms, photographs, correspondence, etc.

The Responsible Agency's Monitoring Manager or Project QA Officer will review all laboratory data and will request additional re-analysis of samples as warranted. Tables 14-1 through 14-3 describe Sampling (Field) QC activities. Tables 14-4, 14-5 and 14-6 describe Analytical QC activities.

Table 14-1. Field Sampling QC (Field Parameters)

Sample Matrix: Water		
<ul style="list-style-type: none"> Sampling SOP: per Monitoring Plan and QAPP Sections 11 and 12 Analytical Parameter(s): Field Parameters Analytical Method/SOP Reference: NA 		
Field QC	Frequency/Number per Sampling Event	Acceptance Limits
Other: Field Measurements	When taking readings, at least 1 minute or longer (if needed) shall be allowed for until stabilization of readings.	See Section 7, Table 7-1

Table 14-2. Field Sampling QC (TSS, Ammonia, Potassium, Surfactants)

Sample Matrix: Water		
<ul style="list-style-type: none"> Sampling SOP: per Monitoring Plan and QAPP Sections 11 and 12 Analytical Parameter(s): TSS, Ammonia, Potassium, Surfactants Analytical Method/SOP Reference: TSS (SM 2540D); Ammonia (SM 4500); Ammonia (EPA 350.1); Potassium (EPA 200.7); Surfactants (SM 5540C) 		
Field QC	Frequency/Number per Sampling Event	Acceptance Limits
Equipment Blanks	1/sample event	< Target reporting limit
Cooler Temperature	4 °C	4 °C
Field Replicate Pairs ¹	5 percent of total number of samples collected per sample event	< 25 percent

¹ Urban Source Evaluation Monitoring Program will not collect field replicates

Table 14-3. Field Sampling QC (*E. coli*, *Enterococcus*, *Bacteroides*)

Sample Matrix: Water		
<ul style="list-style-type: none"> Sampling SOP: per Monitoring Plan and QAPP Sections 11 and 12 Analytical Parameter(s): <i>E. coli</i>, <i>Enterococcus</i>, <i>Bacteroides</i> Analytical Method/SOP Reference: <i>E. coli</i> (EPA 1603, SM 9223B, IDEXX 18HR); <i>Bacteroides</i> (presence/absence <i>Bacteroides thetaiotaomicron</i>)¹ 		
Field QC	Frequency/Number per Sampling Event	Acceptance Limits
Equipment Blanks	1/ sample event	No detectable amounts or < 1/5 of sample concentration
Cooler Temperature	4 °C	4 °C
Field Replicate Pairs ²	5 percent of total number of samples collected per sample event	< 25 percent

¹ Analytical reference for *Bacteroides thetaiotaomicron*: Sauer E.P., J.L. VandeWalle, M.J. Bootsma, and S.L. McLellan. 2011. *Detection of Human Specific Bacteroides Genetic Marker Provides Evidence of Widespread Sewage Contamination of Stormwater in the Urban Environment*. Water Research 45 (2011) 4081-4091.

² Urban Source Evaluation Monitoring Program will not collect field replicates

Table 14-4. Laboratory analytical QC (TSS, Ammonia, Potassium, Surfactants)

Sample Matrix: Water		
<ul style="list-style-type: none"> Sampling SOP: per Monitoring Plan and QAPP Sections 11 and 12 Analytical Parameter(s): TSS, Ammonia, Potassium, Surfactants Analytical Method/SOP Reference: TSS (SM 2540D); Ammonia (SM 4500); Ammonia (EPA 350.1); Potassium (EPA 200.7); Surfactants (SM 5540C) 		
Laboratory QC	Frequency/Number	Acceptance Limits
Method Blank	1/20 samples or 1/batch	< Target Reporting Limit
Laboratory Duplicate	1/20 samples or 1/batch	< 25 percent
Laboratory Matrix Spike	1/20 samples or 1/batch	80 - 120
Matrix Spike Duplicate	1/20 samples or 1/batch	80 – 120; RPD < 25 percent

Table 14-5. Laboratory analytical QC (*E. coli*, *Enterococcus*, *Bacteroides* - water)

Sample Matrix: Water		
<ul style="list-style-type: none"> Sampling SOP: per Monitoring Plan and QAPP Sections 11 and 12 Analytical Parameter(s): <i>E. coli</i>, <i>Enterococcus</i>, <i>Bacteroides</i> Analytical Method/SOP Reference: <i>E. coli</i> (EPA 1603); <i>E. coli</i> (SM 9223B); <i>E. coli</i> (IDEXX 18 hour); <i>Enterococcus</i> (EPA 1600); <i>Enterococcus</i> (IDEXX Enterolert); Genetic markers for human and canine (<i>Bacteroides thetaiotaomicron</i>¹), horse (<i>Bacteroides spp.</i>), bird (<i>Catelliboccus</i>), and rumen (<i>Prevotella</i>). 		
Laboratory QC	Frequency/Number	Acceptance Limits
Method Blank	1/lot minimum	No detectable amounts
Laboratory Duplicate	10 percent of samples or one sample per test run	< 3.27R
Laboratory Control sample (Accuracy)	For each lot of medium received from manufacturer or prepared in laboratory	Verify appropriate response by testing with known positive and negative control cultures for the organism(s) under test

¹ Analytical Method reference for *Bacteroides thetaiotaomicron*: Sauer E.P., J.L. VandeWalle, M.J. Bootsma, and S.L. McLellan. 2011. *Detection of Human Specific Bacteroides Genetic Marker Provides Evidence of Widespread Sewage Contamination of Stormwater in the Urban Environment*. Water Research 45 (2011) 4081-4091.

Table 14-6. Laboratory analytical QC (*E. coli*, *Bacteroides* - sediment/biofilm)

Sample Matrix: Sediment/Biofilm		
<ul style="list-style-type: none"> • Sampling SOP: per Monitoring Plan and QAPP Sections 11 and 12 • Analytical Parameter(s): <i>E. coli</i> • Analytical Method/SOP Reference: <i>E. coli</i> (EPA 1603); Genetic markers for human and canine (<i>Bacteroides thetaiotaomicron</i>¹), horse (<i>Bacteroides spp.</i>), bird (<i>Catelliboccus</i>), and rumen (<i>Prevotella</i>). 		
Laboratory QC	Frequency/Number	Acceptance Limits
Method Blank	1/lot minimum	No detectable amounts
Laboratory Duplicate	10 percent of samples or one sample per test run	< 3.27R
Laboratory Control sample (Accuracy)	For each lot of medium received from manufacturer or prepared in laboratory	Verify appropriate response by testing with known positive and negative control cultures for the organism(s) under test

15. Instrument/Equipment Testing, Inspection, and Maintenance

All laboratories used to implement the SAR Bacteria Monitoring Plan will operate using QA/QC programs to maintain their equipment in accordance with their SOPs, which include those specified by the manufacturer and those specified by the analytical method. Laboratories are required to submit a copy of their SOPs for laboratory equipment maintenance to the QA Officer for review and approval (see Appendices to this QAPP for the SOPs of laboratories being used by this project).

Instruments used to gather field measurements (temperature, conductivity, dissolved oxygen, pH and turbidity) will be properly maintained and calibrated per the manufacturers requirements (Table 15-1). Instruments will be tested prior to the start of field sampling to verify that each instrument is operating appropriately. If the instrument fails to operate within appropriate parameters the Responsible Agency's Project Manager in collaboration with the Monitoring Manager will take the appropriate steps to ensure that the equipment is repaired or replaced in a timely manner.

Table 15-1. Testing, inspection, maintenance of sampling equipment and analytical instruments

Equipment / Instrument	Maintenance Activity, Testing Activity or Inspection Activity	Responsible Person	Frequency	SOP Reference
Multi-parameter Probe	Maintenance and Calibrations	Responsible Agency Monitoring Manager	<ul style="list-style-type: none"> ■ <u>Maintenance</u> – conducted per manufacturer (mfg) specifications; ■ <u>Calibrations</u> - prior to each sampling activity 	Per manufacturer specifications
Marsh McBirney Model 2000 flow meter	Maintenance and Calibrations	Responsible Agency Monitoring Manager	<ul style="list-style-type: none"> ■ <u>Maintenance</u> – conducted per mfg specifications; ■ <u>Calibrations</u> - prior to each sampling activity 	Per manufacturer specifications
Laboratory analytical instruments for Conventional Constituents	Maintenance and Calibrations	Contract Laboratory Personnel	<ul style="list-style-type: none"> ■ <u>Maintenance</u> – conducted per mfg specifications; External calibration with 3 – 5 standards covering the range of sample concentrations prior to sample analysis. At low end, the lowest standard at or near the MDL. Linear regression $r^2 < 0.995$ ■ <u>Calibrations</u> - verification every 20 samples after initial calibration. Standard source different than that used for initial calibration. Recovery 80% - 120%. 	Per individual laboratory SOP manual and per equipment maintenance specifications

16. Instrument/Equipment Calibration and Frequency

All contract laboratories will implement QA/QC programs to calibrate their equipment in accordance with their SOPs, which include those specified by the manufacturer and those specified by the method. Contract laboratories are required to submit a copy of their SOPs for laboratory equipment calibration to each Responsible Agency's Project QA Officer for review and approval (see Appendices to this QAPP for the SOPs of laboratories being used by this project).

A Horiba or similar multi-parameter probe will be used to make field measurements for conductivity, dissolved oxygen, pH, temperature, and turbidity (a Hach turbidimeter may be used to measure turbidity). The instruments will be properly calibrated according to manufacturer specifications prior to each use.

A Marsh-McBirney Model 2000 flow meter will be used to make flow measurements. It will be properly calibrated according to manufacturer specifications prior to each use (see Table 15-1).

17. Inspection/Acceptance of Supplies and Consumables

Contract laboratories will supply all the sample containers necessary for the monitoring program. Other consumable supplies such as latex gloves, plastic storage bags, and waterproof pens will be provided by the Responsible Agency's Monitoring Manager or an appropriate designee (Table 17-1).

All laboratories will implement QA/QC programs to calibrate their equipment in accordance with their SOPs, which include those specified by the manufacturer and those specified by the method. Contract laboratories are required to submit a copy of their SOPs for laboratory equipment calibration to each Responsible Agency's Project QA Officer or its designee for review and approval (see Appendices to this QAPP for the SOPs of laboratories being used by this project).

Table 17-1. Inspection/acceptance testing requirements for consumables and supplies

Project-Related Supplies / Consumables	Inspection / Testing Specifications	Acceptance Criteria	Frequency	Responsible Individual
Sample bottles	Check integrity of bottles; check for preservatives (<i>E. coli</i> , <i>Enterococcus</i>)	Ensure no cracks, intact bottle caps; preservative present	Prior to sample collection	Sampling Personnel
Sample bags	Look for tears/holes	Intact, no tears	Prior to sample collection	Sampling Personnel
Test strips	Check test strips for dampness, evidence of color change	Test strips are dry; no color change noted	Prior to use	Sampling Personnel
Colorimetric test kits	Presence/absence of all chemicals and test solutions	Ensure all necessary chemicals are in the kit	Prior to going to field	Sampling Personnel
Latex gloves	Look for tears/holes	Intact, no tears	Prior to use	Sampling Personnel
Storage bags, pens	Presence/absence of supplies	Ensure supplies are in field bin	Prior to going to field	Sampling Personnel

18. Non-Direct Measurements (Existing Data)

18.1 Data Sources and Uses

During the course of the implementation of the Regional and TMDL Monitoring Programs previously existing relevant water quality and flow data from the monitoring sites will be gathered and stored by the Project Director.

As required to meet program reporting requirements, water quality analyses will be periodically conducted by the Project Director to evaluate water quality data collected from monitoring sites. At a minimum, water quality data collected under the SAR Bacteria Monitoring Plan will be evaluated to determine the following:

- Compliance with applicable water quality objectives for REC1;
- Compliance with applicable antidegradation targets for waters classified as REC2 only;
- Progress towards achieving attainment of MSAR Bacteria TMDL numeric targets for *E. coli*; and
- Impairment status of waterbodies listed as impaired in the watershed but a TMDL has not been adopted.

As part of the effort to evaluate the above, water quality analyses will include descriptive statistics such as geometric mean and percentile calculations. In addition where appropriate, water quality results may be compared to historical data to assess temporal trends at monitoring sites. Descriptive data for each of the monitoring sites has been established in the SAR Bacteria Monitoring Plan (see Attachments A and C in the Monitoring Plan). These data are used by field sampling personnel to determine exact sample collection locations and provide information regarding how to best access the site.

18.2 Data Acceptability

Existing data are considered acceptable for inclusion in data analyses to support the purposes of this the monitoring program only if it meets the following criteria:

- Data was collected with an approved QAPP;
- The sampling methodology and timing are functionally equivalent, including the method for collecting the water samples and the timing of sample collection (e.g., collection during dry versus wet weather or collection from baseflows vs. storm flows); and
- The laboratory analysis methods are functionally equivalent.

Other existing data may be reviewed and discussed to provide additional waterbody or watershed information (e.g., data collected by entities other than those approved in this QAPP), but the use of the data is for qualitative purposes only and will not be incorporated into quantitative data analyses. If these data are used, the constraints associated with the use and interpretation of the data will be described.

19. Data Management

Data will be maintained as described in Section 9 (Documents and Records). During each sample year, each Responsible Agency's Project Manager will maintain an inventory of data and its forms, and will periodically check the inventory against the records in their possession. Data checks (which may be completed by the Monitoring Manager or the Project QA Officer) include:

- Samples are collected according to the procedures outlined in Section 10 (Sampling Process Design).
- Field measurements are recorded on standard Field Log forms included as Attachment 1. Analytical samples are transferred to a contract laboratory under required COC procedures using a standard COC form included as Attachment 2.
- For any site where a velocity cross section profile flow measurement is taken, standard forms are being used to record necessary measurements (Attachment 3).

All laboratory and field data submitted to the Project Director for upload into CEDEN will follow the guidelines and formats established by SWAMP

http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml.

All contract laboratories will maintain a record of transferred records and will periodically assess their record of transferred records against those actually held by a Responsible Agency or the Project Director. Prior to submittal of data by a Responsible Agency to the Project Director, a QA/QC review of the data will be conducted by the Responsible Agency's Data Manager. When all data within a batch set (sample year) have passed QA/QC requirements, the Responsible Agency will submit the data to the Project Director for use in completing the Annual Report. A unique batch number, date loaded, originating laboratory, and the person who loaded the data will be recorded by the Project Director, so that data can be identified and removed in the future if necessary.

The Project Director will compile all data received from Responsible Agencies into a single project spreadsheet/database (annual master dataset). Prior to uploading the annual master dataset into CEDEN as a batch set, the Project Director will conduct an additional final QA/QC review of the data received from each Responsible Agency. The QA/QC review is conducted to:

- Ensure the completeness of the data for the prior sample year;
- Verify the validity of analytical methods, monitoring sites, and sample dates; and
- Ensure that monitoring site information is correctly referenced and that identifiers and descriptions match those provided in the SAR Bacteria Monitoring Plan and this QAPP.

The QA/QC review process implemented by a Responsible Agency or Project Director may involve using automated data checking tools, which assess that new data to be uploaded for consistency with specified rules, including rules that check alpha-numeric formatting, units of measurement, missing information, and others. Data not passing this QA/QC review will be returned to the originating contract laboratory or generator (e.g., a Responsible Agency) for clarification and or correction. Any changes made by the Project Director of data provided by a Responsible Agency will be noted in the annual master dataset.

Responsible Agencies are responsible for ensuring their annual sample year field and laboratory data electronic files are backed-up on a regular basis per the procedures/processes established by their respective agencies. While the Project Director will annually upload the previous sample year's field and laboratory data to CEDEN, the Project Director will maintain a local backup of all electronic files uploaded to CEDEN.

Data submittals from Responsible Agencies to the Project Director will occur by January 15 (dry weather samples) and April 15 (wet weather event samples) of each year and include all data collected in the previous sample year. The Project Director will upload data into CEDEN one time each year within 30 days of submittal of the Final Annual Report.

Group C: Assessment and Oversight

20. Assessments & Response Actions

Data reviews will occur prior to the preparation of the Annual Report (see Section 21). These reviews will be conducted by each Responsible Agency's Project QA Officer and Project Manager. Periodic reviews will always include review of the data to be entered into the SWAMP compatible database to evaluate data accuracy and completeness. Where appropriate, e.g., situations where the laboratory results frequently suggest data quality concerns, audits of laboratory or field sampling teams will be scheduled and conducted. The Santa Ana River Watershed Bacteria Monitoring Program Annual Report will include a data quality assessment section, which will provide documentation of any identified data quality concerns.

If an audit discovers any discrepancy, the Responsible Agency's Project Manager and Project QA Officer will discuss the observed discrepancy with the Monitoring Manager. The discussion will begin with whether the information collected is accurate, what were the cause(s) leading to the data discrepancy, how the deviation might impact data quality, and what corrective actions might be considered.

The Responsible Agency's Project Manager and/or Project QA Officer have the power to halt all sampling and analytical work by field sample teams or contract laboratories if the data discrepancies noted are considered detrimental to data quality. Alternatively, a Project QA Officer can require that certain corrective actions be made within a defined time schedule. This approach may be used as a means to meet the monitoring schedule presented in Section 10.

If sampling work is halted for any reason, the Responsible Agency's Project Manager shall notify the Project Director and the Santa Ana Water Board Project Manager of the issue(s) and expected resolution – both approach and schedule.

21. Reports to Management

21.1. Periodic Reporting

Responsible Agency Project Managers may periodically share data and results from preliminary analyses from Priority site sampling efforts conducted under the SAR Bacteria Monitoring Plan. Other data collected under the SAR Bacteria Monitoring Plan, e.g., specialized studies, may be shared as well.

21.2 Annual Report

The Project Director will be responsible for the development of the Draft and Final Annual Reports and submittal of the Final Annual Report to the Santa Ana Water Board. After the completion of dry weather sampling each sample year (generally May 1 through November 30, see Section 10), the Project Director will send out a reminder to each Responsible Agency and Contract Laboratory to submit all program-related information described above to the Project Director by January 15th. After the completion of wet weather event sampling that will occur each sample year sometime between November 1 and March 31, the Project Director will send out a reminder to each Responsible Agency and Contract Laboratory to submit all program-related information described above to the Project Director by April 15th.

Under the SAR Bacteria Monitoring Program, the Project Director will prepare a Draft Annual Report by April 30th of each year to reflect findings from sampling conducted during the previous sample year (May 1 through April 30). Findings will include a presentation of the data results and any data analyses completed, e.g., descriptive statistics or trend analyses (see Section 7.3). Each Annual Report will include (a) findings from all RMP sites (See Section 3.3); and (b) findings from any additional required monitoring conducted to support implementation of a bacteria TMDL (e.g., see Section 4.1.1.2).

At a minimum, the Draft Annual Report will be submitted to each Responsible Agency and the Santa Ana Water Board for review. A Final Annual Report will be prepared based on the comments received on the Draft Annual Report. The Final Annual Report will be submitted electronically to each Responsible Agency and the Santa Ana Water Board by June 30th of each year. The Final Annual Report will be made available to the public on either the Santa Ana Water Board or Project Director's website.

Group D: Data Validation and Usability

22. Data Review, Verification, and Validation Requirements

Data generated by project activities will be reviewed by each Responsible Agency's Project QA Officer against the data quality objectives cited in Section 7 and the QA/QC practices cited in Sections 14, 15, 16 and 17. Data validation will be performed for each indicator regardless of waterbody. Data validation protocols are presented in Section 23 of this QAPP.

Data will be separated into three categories: (1) Data meeting all data quality objectives; (2) data failing precision or recovery criteria; and (3) data failing to meet accuracy criteria. Data meeting all data quality objectives, but with failures of QA/QC practices will be set aside until the impact of the failure on data quality is determined. Once determined, the data will be moved into either the first or last category.

Data falling in the first category are considered usable by the project. Data falling in the last category are considered not usable. Data falling in the second category will have all aspects assessed. If sufficient evidence is found supporting data quality for use in this project, the data will be moved to the first category, but will be flagged with a "J" as per EPA specifications.

23. Verification and Validation Methods

All data recorded in the field including field measurements, observations, and COC will be checked visually by each Responsible Agency's Project QA Officer and recorded as checked by initials and dates. Field data will be checked to ensure that all necessary data and activities were completed; including collection of all water samples, field blanks, and field replicates, correct units of measurement are reported and values fall within expected ranges. The validation will also check to ensure that samples were delivered to laboratories within required holding times and that all sample handling and custody protocols were followed.

In addition to field data validation, there will be a validation of water quality analysis results. This will involve a review of 10 percent of all laboratory water quality analysis reports. The review will involve verifying that all required parameters were measured, reported in the correct units, and that results fall within expected ranges.

Each Responsible Agency's Project Manager will be responsible for all field data validation reviews. Each of the Laboratory QA Officers will perform checks of all of its records and each of the contract Laboratory Directors will recheck 10 percent. All checks by the laboratories will be reviewed by each Responsible Agency's Project QA Officer and Project Manager.

Issues, including missing data, incomplete site visits, reporting errors (such as incorrect units of measure or incorrect date/time information, etc.), or data management errors will be communicated to the responsible party immediately and documented in the Annual Report for either field sampling, laboratory activities, or database management. If reconciliation and correction of the data are necessary, this will be done through coordination with the Project Director. Any corrections require a unanimous agreement that the correction is appropriate.

24. Reconciliation with User Requirements

The purposes of the Regional and TMDL Monitoring Programs addressed by this QAPP are described in the following sections.

24.1 Regional Monitoring Program

The primary basis for the establishment of a RMP is to evaluate compliance with bacterial indicator water quality objectives established in the Basin Plan for inland freshwaters. The BPA (see Section 5 of this QAPP and SAR Bacteria Monitoring Plan) that established these objectives also established minimum monitoring requirements for the RMP. The RMP is structured to direct water quality monitoring resources to the highest priority waterbodies. As such, the RMP is designed to:

- Provide the data needed to determine if water quality is safe when and where people are most likely to engage in water contact recreation.
- Facilitate the TMDL implementation process and track progress toward attainment of applicable water quality standards, where water quality is impaired due to excessive bacterial indicator levels.
- Apply a risk-based implementation strategy to allocate public resources in a manner that is expected to produce the greatest public health benefit.

With these considerations in mind, priority waterbodies for monitoring under this RMP are described as follows:

- *Priority 1:* The first priority is to establish a monitoring program that can determine whether bacteria levels are "safe" at those locations and seasons where people are most likely to engage in water contact recreation. The Santa Ana Water Board identified these waterbodies in the 2012 BPA as Tier A waters.
- *Priority 2:* The second priority is to focus monitoring resources on those waterbodies that have been identified as "impaired" due to excessive bacterial indicator concentrations and a TMDL has already been adopted. Monitoring efforts to evaluate progress toward attainment with the water quality standard in these impaired waters fall with priority two. This will ensure that the RMP is closely coordinated with TMDL-related sampling efforts.
- *Priority 3:* The third priority is 303(d)-listed or impaired waterbodies where a TMDL has not yet been developed. For these Priority 3 sites the RMP includes periodic sample collection on an annual basis.
- *Priority 4:* The fourth priority is to collect the bacteria indicator data needed to implement the antidegradation targets that have been established for waterbodies designated as REC2 only (i.e., the REC1 beneficial use has been de-designated through an approved UAA). Data collection from these Priority 4 waterbodies provides the Santa Ana Water Board with the ability to assess the status and trend of bacterial indicator water quality as part of the normal Triennial Review process.

24.2 TMDL Monitoring Programs

24.2.1 Watershed-wide Compliance Monitoring

The MSAR Bacteria TMDL required the establishment of a watershed-wide compliance monitoring program to measure compliance with numeric targets established by the TMDL, which were derived from Basin Plan objectives established to protect the REC1 beneficial use. Dry weather monitoring to assess compliance with the MSAR Bacteria TMDL during dry weather has been incorporated into the RMP as Priority 1 or 2 sites.

Wet weather monitoring for bacterial indicators is a requirement of the MSAR Bacteria TMDL. The same concentration based wasteload and load allocations for *E. coli* in the TMDL apply to wet weather as well as dry weather conditions. The Monitoring Plan targets sampling one storm event per wet season to meet this TMDL monitoring requirement.

24.2.2 Urban Source Evaluation Monitoring Program

The purpose of the Urban Source Evaluation Monitoring Program is to identify specific activities, operations, and processes in urban areas that contribute bacterial indicators to waterbodies under the MSAR Bacteria TMDL. This monitoring program also seeks to identify which waters are of greatest concern with regards to the source of the bacteria. Sites where human sources of bacteria are most commonly observed have the highest priority for the implementation of source controls and/or additional monitoring efforts to further refine the identification of sources.

Source evaluation activities in major MS4 drainage areas (Tier 1 sites) and at outfalls within prioritized MS4 drainage areas (Tier 2) have been conducted in 2012-14, and have successfully identified and where possible mitigated controllable sources of bacterial indicator derived from discharges covered by MS4 permits. Continued implementation of source evaluation activities, as needed, is a component of the TMDL Monitoring Program and integral to achieving compliance with the TMDL.

24.2.3 Agricultural Source Evaluation Monitoring Program

The purpose of the AgSEMP is to identify specific activities, operations and processes in agricultural areas that contribute bacterial indicators to MSAR watershed waterbodies. Monitoring data is then intended to be used by the Santa Ana Water Board and agricultural stakeholders to support development of the BASMP. Per the TMDL, the BASMP should include, plans and schedules for the following:

- Implementation of bacteria indicator controls, BMPs and reduction strategies designed to meet load allocations;
- Evaluation of effectiveness of BMPs; and
- Development and implementation of compliance monitoring program(s).

Monitoring downstream of agricultural lands was conducted during wet weather in the 2008-2009 wet season from four monitoring sites and included collection of field parameters, bacterial indicator data, and microbial source identification analyses.

A BASMP is currently under development by agricultural dischargers in the MSAR watershed. Because this document is still under development, this section may be updated once the BASMP is finalized.

Moreover, the final BASMP may include monitoring requirements designed to support implementation of the BASMP. If included in the final program, then these monitoring requirements may be incorporated into the Santa Ana River Watershed Bacteria Monitoring Plan and QAPP.

ATTACHMENT 1

**SANTA ANA RIVER WATERSHED BACTERIA MONITORING PROGRAM
FIELD DATA SHEET FORMS**

Santa Ana River Watershed Bacteria Monitoring Program - Field Data Sheet

General Information:

Site Name: _____
 Site ID: _____
 Date: ____/____/____
 Time (24-hr clock): _____
 Sampling Team: _____/_____

Field Measurements: (average of three readings)

	Reading #1	Reading #2	Reading #3	Average
Conductivity: mS/cm <input type="checkbox"/> μ S/cm <input type="checkbox"/>	_____	_____	_____	_____
Dissolved Oxygen: (mg/L)	_____	_____	_____	_____
pH:	_____	_____	_____	_____
Turbidity: (NTU)	_____	_____	_____	_____
Temp (water): (°C)	_____	_____	_____	_____
Other: _____	_____	_____	_____	_____

Flow Connectivity: Y/N (Describe) _____

Flow measurements (check boxes for units of measure):

Total Section Width (W): _____ feet ☐ meters ☐

Cross-section:	Depth (D)	Velocity (V)	Comments
10% across _____ in <input type="checkbox"/> cm <input type="checkbox"/>	_____ ft/sec <input type="checkbox"/> m/sec <input type="checkbox"/>	_____	
50% across _____ in <input type="checkbox"/> cm <input type="checkbox"/>	_____ ft/sec <input type="checkbox"/> m/sec <input type="checkbox"/>	_____	
90% across _____ in <input type="checkbox"/> cm <input type="checkbox"/>	_____ ft/sec <input type="checkbox"/> m/sec <input type="checkbox"/>	_____	

Estimated Flow _____ ft³/sec ☐ m³/sec ☐ $Q \text{ (ft}^3/\text{sec)} = (0.2 \cdot W \cdot D_{10}/12 \cdot V_{10}) + (0.6 \cdot W \cdot D_{50}/12 \cdot V_{50}) + (0.2 \cdot W \cdot D_{90}/12 \cdot V_{90})$

Grab Sampling:

Filled and labeled (check if applicable)

1 – 100 mL or 125 mL polyethylene bottle (w/ NaSO₄ preservative) for ***E. coli* or *Enterococcus***:
 1 – 1,000 mL polyethylene bottle for **TSS**:
 1 – 1,000 mL polyethylene bottle for ***Bacteroides***:
 1 – 100 mL polyethylene bottle for **Ammonia**:
 1 – 500 mL polyethylene bottle for **Potassium**:
 1 – 500 mL polyethylene bottle for **Surfactants**:
 Additional bottle sets are included for field duplicates and trip blanks

Site Observations:

Weather: _____
 Visual Evidence of REC-1 Activity: _____

 Other: _____

ATTACHMENT 2

SANTA ANA RIVER WATERSHED BACTERIA MONITORING PROGRAM
EXAMPLE CHAIN OF CUSTODY FORMS



County of Orange, Health Care Agency
 Water Quality Laboratory (ELAP # 2545)
 600 Shellmaker Rd. Bldg. A
 Newport Beach, CA 92660
 Phone:(949)219-0423 FAX:(949)219-0426

Client: **OCPW**
 Study/Billing Code: Santa Ana Regional Monitoring Program
 Contact Info:
 Date Collected: mm/dd/yyyy
 Sampler Name: County of Orange

Bottle #	Time Collected	MRN/Station ID, Location	Sampler Comments	Submitter Accession #	Lab Accession #	Test Requested
1		*1000-34-2417* Site1Name	Matrix: FW W: Ht: Q: FTO: Temp:	WRzzzzzz Notes:		Total Coliform
						Fecal Coliform
						Enterococci
						<input checked="" type="checkbox"/> E. coli
						Can Bacrio
						Hum Bacrio
						Coliphage
Water Type: Domestic Surface Marine Ground Reclaimed Other Preservative: Na ₂ S ₂ O ₃ None Other						
2		*1000-34-2418* Site2Name	Matrix: FW W: Ht: Q: FTO: Temp:	WRzzzzzz Notes:		Total Coliform
						Fecal Coliform
						Enterococci
						<input checked="" type="checkbox"/> E. coli
						Can Bacrio
						Hum Bacrio
						Coliphage
Water Type: Domestic Surface Marine Ground Reclaimed Other Preservative: Na ₂ S ₂ O ₃ None Other						
3		*1000-34-2418* Site3Name	Matrix: SW W: Ht: Q: FTO: Temp:	WRzzzzzz Notes:		Total Coliform
						Fecal Coliform
						Enterococci
						<input checked="" type="checkbox"/> E. coli
						Can Bacrio
						Hum Bacrio
						Coliphage
Water Type: Domestic Surface Marine Ground Reclaimed Other Preservative: Na ₂ S ₂ O ₃ None Other						
4		*1000-21-6890* Site4Name	Matrix: FW W: Ht: Q: FTO: Temp:	WRzzzzzz Notes:		Total Coliform
						Fecal Coliform
						Enterococci
						<input checked="" type="checkbox"/> E. coli
						Can Bacrio
						Hum Bacrio
						Coliphage
Water Type: Domestic Surface Marine Ground Reclaimed Other Preservative: Na ₂ S ₂ O ₃ None Other						
Water Type: Domestic Surface Marine Ground Reclaimed Other Preservative: Na ₂ S ₂ O ₃ None Other						
Name / Date / Time				Comments:		
Relinquished by:						
Received by:						
Analyzed by:						
Reviewed by:						
Reported to:				Transport Conditions:		

ORANGE COUNTY WATER DISTRICT

10500 Ellis Avenue, Fountain Valley, CA 92708

Telephone: (714) 378-3200 Fax: (714) 378-3373

CHAIN OF CUSTODY RECORD

NO.	SAMPLING AGENCY	WRMS STATION NAME	Sample Date	Sample Time	Sampled BY	COMMENTS			NO. OF Bottles	ANALYSIS
						EC=	Ph=	DO=		
1						EC=	Ph=	DO=		
						TEMP=				
						EC=	Ph=			
2						TEMP=	DO=			
						EC=	Ph=			
						TEMP=	DO=			
3						EC=	Ph=	DO=		
						TEMP=				
						EC=	Ph=	DO=		
4						TEMP=	DO=			
						EC=	Ph=			
						TEMP=	DO=			
5						EC=	Ph=	DO=		
						TEMP=				
						EC=	Ph=	DO=		
6						TEMP=	DO=			
						EC=	Ph=			
						TEMP=	DO=			
7						EC=	Ph=	DO=		
						TEMP=				
						EC=	Ph=	DO=		
8						TEMP=	DO=			
						EC=	Ph=			
						TEMP=	DO=			
9						EC=	Ph=	DO=		
						TEMP=				
						EC=	Ph=	DO=		
10						TEMP=	DO=			

RELINQUISHED BY:

DATE/TIME

ED BY:

RELINQUISHED BY:

DATE/TIME

ED BY:

SPECIAL INSTRUCTIONS:

BILL ACCOUNT NO.:

DATE/TIME

Chain of Custody Sample Information Record

Page _____ of _____

ATTACHMENT 3

SANTA ANA RIVER WATERSHED BACTERIA MONITORING PROGRAM
FLOW MEASUREMENT FORM

SANTA ANA RIVER WATERSHED BACTERIA MONITORING PROGRAM

FLOW MEASUREMENTS										
Portable Flowmeter Used _____								Location _____		
Left Bank _____ Right Bank _____								Recorder _____		
								Date _____		
								Time _____		
								Page _____ of _____		
	Distance from IP	Width	Total Depth	Flow Velocity				Average V*	Area A**	Discharge (avg VXA)
				VO.6	VO.2	VO.8	VO.9			
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
Total Discharge										
<p>Stream Flow Conditions (I.e., muddy, clear, debris, etc...): _____</p> <p>_____</p> <p style="margin-top: 20px;">* Average Velocity =VO.6 for stream depths between 0.3 and 2.5 feet (six-tenths method). =(VO.2 + VO.8)/2 for stream depths greater than 2.5 feet (two-point method). =VO.9 if flow is less than 0.3 feet deep (maximum velocity X 0.9).</p> <p style="margin-left: 40px;">** Area =total depth x width IP =Initial Point</p>										

APPENDICES

APPENDIX A

**ORANGE COUNTY PUBLIC HEALTH WATER QUALITY LABORATORY
STANDARD OPERATING PROCEDURES**

ENVIRONMENTAL LABORATORY OPERATION PROGRAM PLAN

Orange County Public Health Laboratory
Water Quality Laboratory



COMPLETED PLAN PREPARED BY:
Joseph Guzman, OCPHL, 2015

Refer correspondence to:

Joseph Guzman, OCPHL
600 Shellmaker Road, Bldg A
Newport Beach, CA 92660

Main: (949) 219-0423
Fax: (949) 219-0426

Approval:

Laboratory Director: Richard Alexander.

Signature: 

Date: 1/28/2015

Environmental Laboratory Operation Program Plan Table of Contents

Certification

Notice of continued certification from California Department of Health Services
List of Orange County Public Health Laboratory (OCPHL) Water Quality Department
Fields of Testing
Copy of ELAP Certificate

1. General Information and Objectives

- 1.1. ELOPP Overview
- 1.2. ELOPP Objectives
- 1.3. ELOPP Overview Check-off Sheet
- 1.4. ELOPP Plan Maintenance and Update Form
- 1.5. Investigation/Discrepancy Report

2. Organization

- 2.1. County of Orange Health Care Agency Organization Chart
- 2.2. OCPHL Organization Chart
- 2.3. OCPHL Water Quality Department Organization Chart
- 2.4. Water Quality Department Floor Plan
- 2.5. Chain of Custody

3. Personnel

- 3.1. Water Laboratory Microbiologist Duties
- 3.2. Water Laboratory Lab Assistant Duties
- 3.3. Principal Analyst Personnel Qualifications
- 3.4. Personnel Training and Competency Record (Blank)

4. Sample Collection, Transport and Disposal

- 4.1. Water Sample Collection SOP
- 4.2. Sample Collection Bottle QC Form (blank)
- 4.3. Sample Receiving SOP
- 4.4. Laboratory Sign-in Sheet (blank)
- 4.5. Water Sample Disposal Procedure

5. Data Reporting

- 5.1. Reporting Test Results SOP
- 5.2. Data Handling Procedure
- 5.3. Membrane Filtration Report Form (blank)
- 5.4. Membrane Filtration Report Form (sample)
- 5.5. Colilert™/Multi-Tube Fermentation Report Form (blank)
- 5.6. Heterotrophic Plate Count Report Form (blank)
- 5.7. Result Notification Log Form (blank)
- 5.8. Laboratory Disposal of Records Memo

6. Laboratory Equipment

- 6.1. Equipment QA Summary
- 6.2. Laboratory Equipment List
- 6.3. Equipment Maintenance/Repair Log form (Blank)
- 6.4. Procedure for Checking Temperatures on Equipment
- 6.5. Monthly Incubator Temperature Log (Blank)
- 6.6. Monthly Waterbath Temperature Log (Blank)
- 6.7. Monthly Refrigerator Temperature Log (Blank)
- 6.8. List of Certified Thermometers
- 6.9. Example of Thermometer Certificate of Calibration
- 6.10. Monthly ELAP Autoclave Validation Checklist (Blank)
- 6.11. Quanti-tray Sealer Performance Check (blank)

7. Glassware

- 7.1. Glassware pH Checklist/SOP (Blank)
- 7.2. Inhibitory Residue Testing SOP
- 7.3. Inhibitory Residue Report Form (Current)
- 7.4. Inhibitory Residue Report Form (blank)

8. Reagent Grade Water

- 8.1. Quality Control of Reagent Grade Water SOP
- 8.2. Quality Control of Milli-Q Water Report Log
- 8.3. Total Chlorine Analysis of Reagent Water SOP
- 8.4. Annual Microbiological Suitability Test of Reagent Grade Water SOP
- 8.5. Report from Annual Microbiological Suitability Test
- 8.6. Quality Control Report form for Buffered Water used in MF
- 8.7. Hardy Diagnostic Reagent Water Specifications
- 8.8. Northeast Lab Continental RO Water System SOP

9. Media and Reagents

- 9.1. Media QC Summary
- 9.2. Media Requisitions:
 - 9.2.1. Brilliant Green Lactose Bile Broth, Buffered Water for Membrane Filtration, EC Medium, Lauryl Tryptose Broth, m-ENDO LES Agar, mEI Agar, mFC Agar, Nutrient Broth, Standard Plate Count Agar
- 9.3. Media QC Sheets:
 - 9.3.1. MF Monthly Media Verification Log, Sample Media QC Sheet
- 9.4. Reagent QC Sheets:
 - 9.4.1. Gram Stain QC, Catalase QC
- 9.5. Media Transport SOP

10. Membrane Filtration

- 10.1. Membrane Filter Manifold Sterility Check-list
- 10.2. Recreational Water Membrane Filtration SOP
- 10.3. Membrane Filtration Media Quality Control SOP

- 10.4. Membrane Filtration Media Quality Control Diagram
- 10.5. Membrane Filtration Workload Log

11. Analytical Quality Control Procedures

- 11.1. Measurement of Method Precision SOP
- 11.2. Method Precision Log Sheet
- 11.3. Analyst Comparison of Plate Counts SOP
- 11.4. Analyst Comparison of Plate Counts Log Sheet
- 11.5. Precision Criterion Worksheet Template
- 11.6. Daily Precision Worksheet Template

12. Parallel Testing

- 12.1. Comparison between MTF and MF (m-Endo)
- 12.2. Comparison between MTF and MF (m-FC)
- 12.3. Comparison between Quanti-tray™ and MF (mEI)
- 12.4. Evaluation of Enterolert™ and mEI Agar for the Enumeration of Enterococci in Environmental Waters

Certification

Continued certification from California Department of Health Services

OCPHL Water Quality Department Fields of Testing (see ELOP Plan)

ELAP Certificate # 2545

1. General Information and Objectives

1.1. ELOP Plan Overview

1) Laboratory facility:

The Water Quality Laboratory (WQL) is currently located in Newport Beach. The current laboratory area includes office space and work areas. It is equipped with refrigerators, incubators, freezers, water baths, microscopes and instruments for use in research projects and routine water analyses.

2) Personnel

- a) Richard Alexander is the director of the Orange County Public Health Laboratory (OCPHL) and the Water Quality Laboratory. The laboratory director is responsible for the operations of the WQL. He is present at the WQL at least once per week. The WQL supervisor attends mandatory supervisor meetings at the OCPHL on a weekly basis to provide operational reports to the Director and other supervisors.
- b) The WQL is currently staffed with two Microbiologists and two Laboratory Assistants, who are supervised by Joseph Guzman. The microbiologists and supervisor are certified Public Health Microbiologists. Personnel qualification forms are filed in the ELOP Plan binder

3) Sample Receiving/Storage

a) Collection Personnel/Sampling and Sample Custodian Instructions

- i) All sampling personnel are trained in the proper sample collection techniques, which are available in the SOP. A sample collection/receiving procedure (see SOP) is available to all sample collection personnel.

b) Sample Receiving

- i) The sample receiving area is located adjacent to the delivery entrance of the WQL. Sample receiving instructions are posted on the sample-receiving table. Included in these instructions is a description of the sample acceptance requirements, including transport times and temperatures, and required information for the report form (Chain of Custody).
- ii) All sampling, receiving, processing, and reading times, sample test requests, sample type, and sample collector information are reported on the sample report form.
- iii) Date, time, number and type of samples, transport temperature, as well as the initials of the sampler and receiver are all reported on the lab sign-in sheet, which is located at the sample receiving table.
- iv) Each sample received is labeled with a Water Lab accession number, which is independent of any previously assigned number but is consecutive in the order received by the water lab.

c) Sample Storage

- i) Samples are stored refrigerated ($\leq 5^{\circ}\text{C}$) until processed. Compliance samples must be processed within 6 hours of collection time.

4) Records/Data Retention

a) Data Retention Requirements

- i) All laboratory records will be retained for a minimum of five years.
 - ii) Chain of custody forms, instrument data, and calibration reports are accessible on-site for a minimum of two years. After two years the records could be moved to a records retention warehouse under the control of the County's Custodian of Records. If sent to the records retention warehouse, all records will be held for a minimum of three years before disposal. Submitting agencies will be given notice of records disposal prior to laboratory records being disposed.
 - iii) Copies of finalized results are available to the submitting agency upon request.
 - iv) All water data is maintained in the CERNER PathNet system, an electronic database.
- b) Raw Data
 - i) All data is entered into the CERNER PathNet system and performed. The calculated results are verified by licensed Public Health Microbiologist or Lab Assistant before they are released to the submitting agency. A record of personnel verifying results is electronically captured.
- c) Corrective actions
 - i) All corrective actions, whether it is pertaining to sample analysis, equipment issues, media quality, or performance analysis, are documented on an 'Investigation/Discrepancy Report' form.
 - ii) All corrective actions pertaining to sample analysis are also noted on the bottom of the water sample report form. The documentation includes: date, analyst, sample affected, problem, and resolution.
- d) Data Reduction
 - i) All significant figure calculations are based on the methods outlined in EPA – *Microbiological Methods for Monitoring the Environment* (1978).
- e) Notification and Reporting Procedures
 - i) OCPHL provides water sample result spreadsheets to the submitting agencies. The sample chain of custody form, along with the lab sign-in sheet are used to meet chain of custody requirements.
 - ii) Reporting of significant figures, detection limits, and report corrections are described in detail in the Membrane Filtration SOP.
 - iii) Results are entered into the CERNER PathNet system and reported as CFU/100 ml or MPN/100ml depending on the analysis method.
 - iv) The sample chain of custody form includes the following information:
 - (1) Laboratory name and ELAP certificate number
 - (2) Complete sample ID including Laboratory Number and submitting agency number designations
 - (3) Sample collector name or initials
 - (4) Date/time of sample collection
 - (5) Sample collection bottle number
 - (6) Station number/location of sampling station
 - (7) Date/time of sample receipt in the laboratory
 - (8) Date/time of sample analysis

- (9) Type of analytical method used
- (10) Date of finalized report
- (11) Initials of lab testing personnel
- 5) Basic Laboratory Instrumentation
 - a) Maintaining properly operating equipment in the Water Lab is of utmost importance. The Forma O₂ incubator temperatures are checked twice daily, with calibrated thermometers on each shelf. The water bath is also checked twice daily, at least four hours apart. Freezers and refrigerators are checked once a day. The temperatures are recorded on a temperature chart specific for each unit. Any temperatures out of range are noted in red and a Microbiologist is notified to take immediate corrective action. All corrective actions are noted on the temperature chart in its appropriate column. The equipment is kept on a monthly primary maintenance schedule contracted out to a private maintenance company. The biological safety cabinet and fume hood is certified by CEPA every six months.
 - b) pH Meter
 - i) The pH meter is maintained and operated by the media room at the laboratory at 1729 W. 17th St., Santa Ana, CA, 92706 (Main Laboratory).
 - c) Analytical Reagents
 - i) Most analytical reagents are bought commercially. The date and initial of person who initially opens the reagent is noted on the reagent bottle. No reagents are used beyond expiration. Reagents are disposed of according to the manufacturer's recommendation.
 - d) Conductivity Meter
 - i) Conductivity of reagent water is determined at the main laboratory, using a 0.01 M KCL calibration solution as stated in Standard Methods 2510B: 3. The conductivity meter is calibrated before each use and results are recorded each time on the Milli-Q QC Report Sheet.
 - e) Analytical Balance
 - i) The analytical balance is located in the media room of the main laboratory. The balance self-calibrates with each use. A contract service company performs annual maintenance and calibration certification. These records are kept in the media room files.
 - f) Refrigerators and Freezers
 - i) Refrigerators and freezers are monitored daily with a calibrated thermometer immersed in liquid. Temperature records are noted with the date, temperature, and initial of the person responsible. Monthly temperature sheets are maintained within the equipment folder for each unit.
 - ii) Primary maintenance of each unit includes cleaning and/or defrosting of the interior monthly or as needed. Temperature calibration on those units with fixed thermometers is performed annually by a contract company.
 - g) Water Baths
 - i) Water bath temperatures are recorded daily. Monthly temperature charts are maintained within the equipment folder for each of the units.

- ii) Primary maintenance includes monthly disinfection and replacement with sterile water. A temperature calibration by a contract company is performed annually.
- h) Thermometers
 - i) All thermometers are calibrated annually. Updated calibration stickers are placed on the thermometer. Calibration certificates are maintained in the thermometer files.
- i) Sample Containers
 - i) All sample collection containers are stored in the manufacturers shipping box. Boxes are kept closed and stored in the storage area. Five bottles from each box are tested for sterility before use.
- 6) Precision, Accuracy and Comparability
 - a) The precision and accuracy of data for water samples is determined following procedures outlined in Standard Methods (SM) and by testing replicate samples. The Measurement of Method Procedure (SM 9020B) is used to determine analyst method precision. For precision of microbiological data for research projects, the standard deviation (SD) of replicate measurements must fall within the 95% confidence interval of the mean plus or minus 1.96 SD, or for few replicates, \bar{X} plus or minus t , where t is the critical value of Students t distribution. Accuracy for water analyses is based upon whole units, which are individual colonies for enumeration and comparison to positive and negative controls. Sampling procedures, testing volumes and standardized analytical methods are used to achieve comparability.
- 7) Performance and System Audits
 - a) Analyst Audits
 - i) Measurement of method precision is performed daily on 10% of samples processed.
 - ii) Analyst comparison of plate counts are performed monthly among all analysts who count plates.
 - b) Proficiency Samples
 - i) Water Lab participates annually in performance evaluation sample studies for each field of testing. Those individuals who routinely perform each test method analyze these sample sets.
- 8) Internal Quality Control
 - a) Internal quality control checks are conducted on a routine basis. The type and frequency of internal quality control checks are detailed in the SOP. Microbiological media and reagents must meet acceptable criteria based on Standard Methods or manufacturer recommendations prior to use. Control samples are included during water analyses.
 - b) Equipment
 - i) Temperature checks are performed, using calibrated thermometers, twice daily on all incubators and waterbaths, and once daily on all freezers and refrigerators.
 - ii) Routine monthly maintenance includes thorough cleaning and disinfection of all internal and external surfaces.

- iii) Primary annual maintenance includes calibration of all internal thermometers, which is performed by an outside contract company.
 - c) Media
 - i) All media with new lot numbers or production dates are checked for proper reactions using quality control organisms. Five percent of all media are set aside for sterility checks.
 - d) Reagents
 - i) All new reagents are tested for proper reactions using quality control organisms.
 - e) Lab ware
 - i) All glassware is tested annually for pH and inhibitory residue (tested with every change in detergent lot number).
- 9) Safety
 - a) Safety Equipment
 - i) Fire Extinguishers are located next to the main entrances, as well as centrally located in-between sample processing stations.
 - ii) An eye wash station is located at each work area sink, and in the hallway by the restrooms.
 - iii) Mercury spill kits are located in the end cabinets of the last two work area benches.
 - iv) First Aid kits are located by the first work area sink and in the storage area.
 - v) Safety glasses are stored in the drawers next to each work area.
 - b) Flammable Liquid Storage
 - i) All flammable liquids are stored in the “flammable cabinet”.
 - c) Hazardous Wastes Handling
 - i) Biological wastes are disposed into red biohazard bags, which are stored in large covered plastic trash receptacles. A certified hazardous waste company picks up and disposes all biohazard refuse.
- 10) Test Method References
 - a) The following reference materials are available for use within the water lab:
 - i) 22nd Edition *Standard Methods for the Examination of Water and Wastewater* (2012)
 - ii) 21st Edition *Standard Methods for the Examination of Water and Wastewater* (2005)
 - iii) 20th Edition *Standard Methods for the Examination of Water and Wastewater* (1998)
 - iv) 18th Edition *Standard Methods for the Examination of Water and Wastewater* (1992)
 - v) EPA – *Health Effects Criteria for Marine Recreational Waters* (1983)
 - vi) EPA – *Handbook for Sampling and Sample Preservation of Water and Wastewater* (1982)
 - vii) EPA – *Microbial Methods for Monitoring the Environment* (1978)
 - viii) EPA – *Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and Escherichia coli* (2000)

1.2. ELOP Plan Objectives

1) Introduction

- a) The Water Quality Laboratory (WQL), located in Newport Beach is an adjunct laboratory of Orange County Public Health Laboratory (OCPHL) located in Santa Ana, California. Microbiological water analyses are performed at the WQL. Chemistry testing on water samples is performed by the Chemistry Section of the OCPHL located in Santa Ana.
- b) OCPHL is committed to ensure data accuracy and quality. The laboratory has been performing indicator bacterial testing according to an established quality assurance program approved by the Environmental Laboratory Accreditation Programs (ELAP) of the California Department of Health Services (DHS) (certificate number 2545). Accreditation is required of an environmental laboratory for producing analytical data for California regulatory agencies. The data may be used to demonstrate compliance with applicable requirements of drinking water and wastewater sections of the California health and Safety and Water codes. The accreditation process includes completion of: (1) verification of a quality assurance program, (2) successful analysis of performance evaluation samples, (3) successful completion of a site visit by an ELAP auditor.
- c) Microbiological water analyses are performed using testing methods in the Standard Methods for the Examination of Water and Wastewater, 20th edition and USEPA Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*. Laboratory personnel follow standard operational procedures (SOPs) for media preparation, sample collection and log-in, analytical methods (including references), data reduction, validation and reporting and Quality Assurance/Quality Control (QA/QC) procedures as established by the OCPHL. Laboratory personnel are required to follow safe laboratory practices and encouraged to participate in continuing education classes. Laboratory instruments and equipment are maintained on a regular basis to verify operation to manufacturer's and laboratory specifications. Instruction manuals and maintenance records for laboratory equipment are kept on file.

2) ELOP Plan

- a) The Water Quality Laboratory ELOP Plan is inclusive of:
 - Certification
 - General Information and Objectives
 - Organization
 - Personnel
 - Data Reporting and Reduction
 - Laboratory Equipment
 - Glassware
 - Reagent Grade Water
 - Media and Reagents
 - Membrane Filtration
 - Analytical Quality Control Procedures

Parallel Testing

- 3) Precision, accuracy and comparability
 - a) The precision and accuracy of data for water samples is determined following procedures outlined in Standard Methods (SM) and by testing replicate samples. The Measurement of Method Procedure (SM 9020B) is used to determine analyst method precision. For precision of microbiological data for research projects, the standard deviation (SD) of replicate measurements must fall within the 95% confidence interval of the mean plus or minus 1.96 SD, or for few replicates, \bar{X} plus or minus t , where t is the critical value of Student's t distribution. Accuracy for water analyses is based upon whole units, which are individual colonies for enumeration and comparison to positive and negative controls. Sampling procedures, testing volumes and standardized analytical methods are used to achieve comparability.
- 4) Data management, analysis, validation and reporting
 - a) Field data samples include the following information: bottle number, time collected, station number/location of sampling station is tabulated on sample chain of custody form at time of sample collection. The date and time of receipt and time that the samples are processed are also recorded on the sample chain of custody form. The results are entered into the CERNER PathNet system and reported as CFU/100 ml or MPN/100ml. Sample chain of custody forms are reviewed and signed by senior staff. Hard copies of the chain of custody forms are maintained in binders and archived for 5 years from the time they are completed. Water results data are available upon request. The data is entered into an electronic database and maintained on a separate disk.
- 5) Internal quality control
 - a) Internal quality control checks to validate laboratory personnel performance are conducted on a routine basis. The type and frequency of internal quality control checks are detailed in the SOP. Microbiological media and reagents must meet acceptable criteria based on Standard Methods or manufacturer recommendations prior to use. Control samples are included during water analyses. Laboratory instruments and equipment are checked prior to use. Proficiency samples obtained from commercial sources are used to validate analyst techniques and practices.
- 6) Personnel
 - a) Richard Alexander is the director of the Orange County Public Health Laboratory and the Water Quality Laboratory. The laboratory director is responsible for the operations of the WQL. He will be present at the WQL at least once per week. The WQL supervisor attends mandatory supervisor meetings at the OCPHL on a weekly basis to provide operational reports to the Director and other supervisors.
 - b) The WQL is currently staffed with 2 microbiologists and 2 laboratory assistants who are supervised by Joseph Guzman. The microbiologists and supervisor are certified Public Health Microbiologists. Personnel qualification forms are filed in the ELOP Plan binder.

7) Laboratory facility

- a) The WQL is located on Shellmaker Island in Newport Beach, CA. The laboratory area includes office space and work areas. It is equipped with refrigerators, incubators, freezers, water baths, microscopes and instruments for use in research projects and routine water analyses.

1.3. ELOPP Overview Check-off Sheet

The Water Laboratory ELOP Plan is updated routinely. In order for all employees to be familiar with any new updates, there is a need for a regular review of ELOPP objectives. The Director, Supervisor, as well as all who work in the Water Quality Lab should review the Water Lab ELOP Plan annually. Please read and pay close attention to any new additions or changes in the procedures. After review of the ELOP Plan, please fill out the chart below with all required information.

Date	ELOPP Change	Reason	Initial

1.4. ELOP Plan Maintenance and Update Form

The Water Laboratory ELOP Plan is updated routinely. After review of any updates by the Supervisor, please fill out the chart below with all required information.

Date	ELOPP Change	Reason	Initial

1.5. Investigation/Discrepancy Report

This is a sample report:

Date: _____

Nature of Discrepancy: _____

Name of Product: _____

Manufacturer: _____

Catalog Number: _____

Expiration Date: _____

Lot Number: _____

Date Ordered: _____

Requisition Number: _____

Follow-up: (Instruction/Action)

Date Called: _____

Called By: _____

Talked to: _____

Telephone #: _____

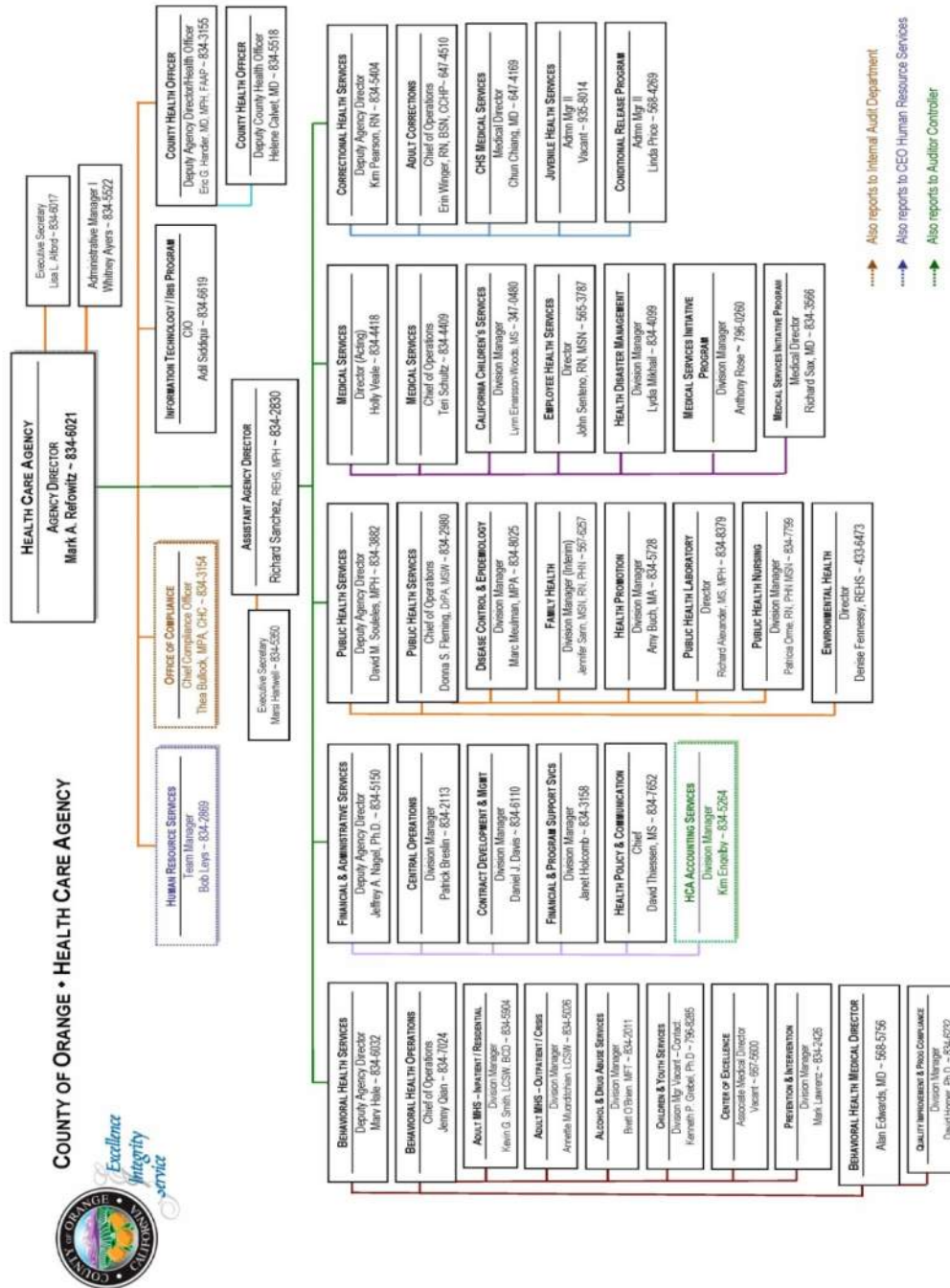
Problems/Comments:

Supervisor Review: _____

Date: _____

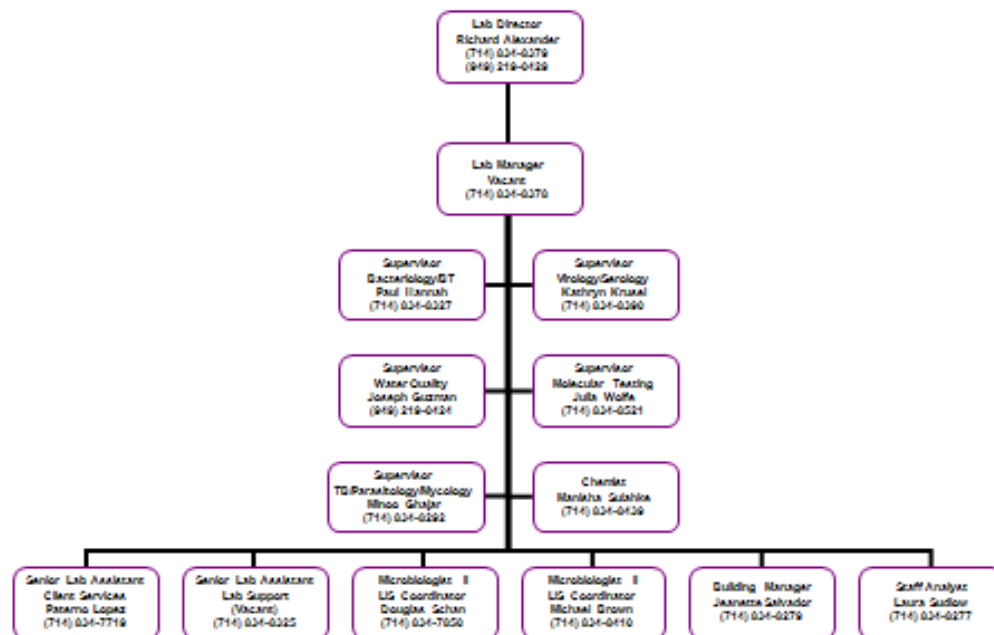
2. Organization

2.1. County of Orange Health Care Agency Organization Chart

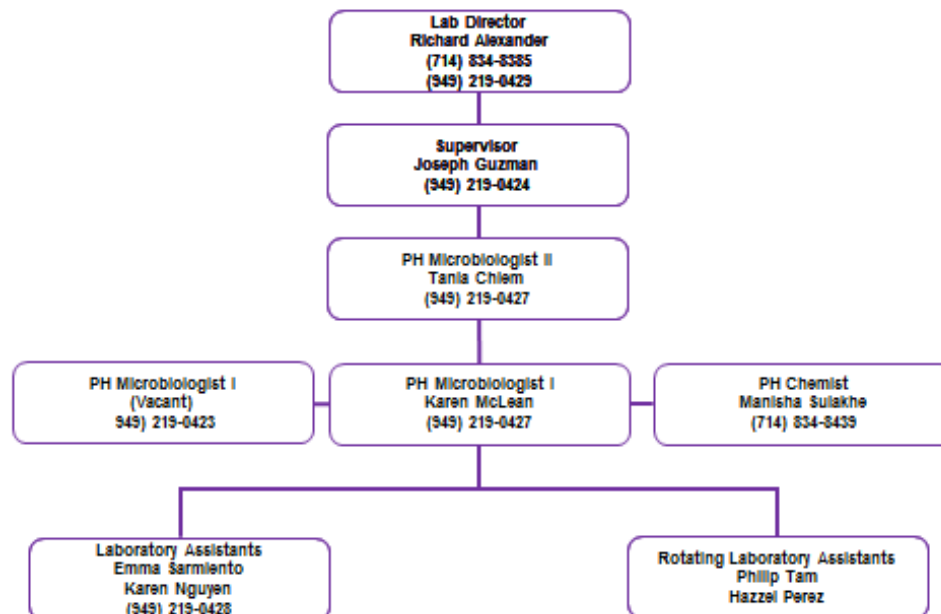


HCA Org Chart January 2014 - J.M.Larwell

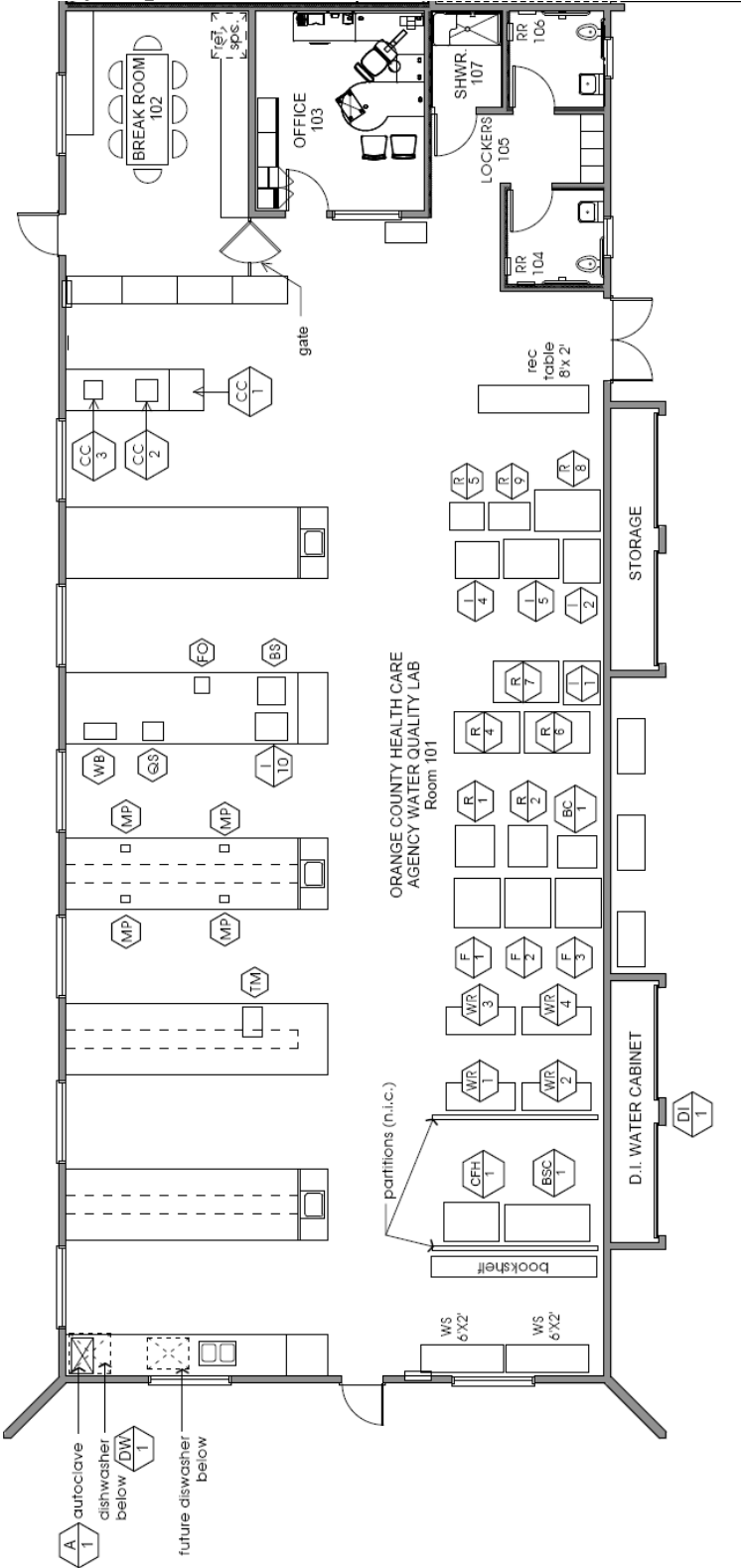
2.2. OCPHL Organization Chart



2.3. OCPHL Water Quality Department Organization Chart



2.4. Water Quality Department Floor Plan



2.5 Chain of Custody

1) LOGGING AND RECEIVING SAMPLES

- a) Use proper safety precautions when handling water samples.
- b) Samples and chain of custody forms must be checked for the following information before the sample can be accepted from the sampler:
 - i) Chain of Custody Form (COC):
 - (1) Date collected
 - (2) Time collected: samples should arrive to the lab within 6hrs after being collected
 - (3) Last name of sampler
 - (4) Type of sample (i.e. marine, domestic, sewage, groundwater, surface water)
 - (5) Location and station number
 - (6) Test(s) requested
 - (7) Cooler temperature must be recorded
 - (8) Date received
 - (9) Arrival time of the sample
 - (10) Initials of both sampler and receiver
 - (11) NOTE: Temperature should be greater than 0°C and less than 10°C.
- c) Using the pre-printed accession labels, number each sample (on COC and sample bottle) with a WL number, consecutively maintaining the numbered order set-up by the sampler. Check the last WL number from previous day before numbering. **DO NOT SKIP ANY NUMBERS!**
- d) Indicate time samples received/run on the COC and initial.

2) PROCESSING SAMPLES

- a) Process compliance samples within 6 hours from collection time. Non-compliance samples may be processed beyond the holding time but no later than 24 hours from the time of collection. Note the processing time on the worksheet.
- b) Refer to posted dilution chart located on receiving table for standard volumes to be tested per submitting agency.

3) READING SAMPLE PLATES

- a) Use dissecting microscope for viewing of typical colonies.
- b) Count the colonies according to procedure outlined in SOP.
- c) Input testing dilutions and counts into the Cerner PathNet System

4) REPORTING

- a) Results are entered into the CERNER PathNet System by the Microbiologist/Lab Assistants on the day that Membrane Filtration plates are read.
- b) All water results are verified by the Microbiologist/Lab Assistant upon completion.
- c) Document any faxed, e-mailed or verbal notification of results in the Comments field of the COC.
- d) File the completed COC in the “Water lab Worksheet” binder.
- e) Follow the instructions listed in the Data Reporting section for each submitter when reporting results.

3. Personnel

3.1. Water Laboratory Microbiologist Duties

- 1) Review water results daily.
- 2) Read and record all Quality Control for water lab media.
- 3) Calculate precision criterion for duplicate analysis on the first 15 positive samples of the same sample type. Enter the results in the computer. This test must be performed by all lab assistants in Water Lab at the beginning of rotation. Thereafter, analysis on one sample from every run, or 10% of daily workload should be setup in duplicate.
- 4) Set up Membrane Filtration verification test on typical and atypical colonies. Use MTF procedures on Gram-negative bacteria. Use an additional short setup of biochemicals for *Enterococcus* species.
- 5) Perform the Annual Inhibitory Residue test.
- 6) Send Milli-Q water to a private laboratory for Annual Testing.
- 7) Participate in ELAP proficiency.
- 8) Record all data in ink with changes lined through such that original entry is visible. Changes are initialed and dated.

3.2. Water Laboratory Lab Assistant Duties

- 1) Read and record temperatures on all equipment.
 - a) For all O₂ incubators, read and record temperature of each shelf twice a day, within a minimum of 4 hours apart.
 - b) Read and record temperature of water baths twice a day. Add water as needed, using sterile de-ionized water.
 - c) If equipment is out of range, record temperature in red ink and report to Microbiologist for corrective action.
 - d) Document corrective action on the temperature chart.
 - e) Change temperature charts monthly.
- 2) Perform inventory for water lab every Monday.
- 3) Log in and process water samples for Membrane Filtration (MF).
- 4) When sample processing is completed, place all pipettes into pipette discard containers. Place sample containers and lids from potentially contaminated specimens into sink with 10% bleach for decontamination.
- 5) Keep record of quality control on media and Milli-Q water.
- 6) Keep Workload log, Notification log, and ELAP Autoclave Validation Checklist updated.
- 7) Perform duplicate analysis on 15 positive samples of same type at the beginning of rotation. Thereafter, setup one a day or 10% of daily samples, whichever is greatest.
- 8) Sub-culture quality control cultures on Monday, Wednesday and Friday, or when required for media QC.
- 9) All water records must be kept for five years. All quality control records and temperature charts are held for two years, and then sent to Records Retention.
- 10) File all media preparation sheets and hold for two years.
- 11) Clean cabinet shelves when empty.

- 12) Clean and dust room weekly. Clean glass doors on incubators.
- 13) Wipe bench tops before and after each use. Clean sink as needed.
- 14) Clean water baths every month. (Use de-ionized water)
- 15) Tally water samples after reports are completed. Print the tally report at the end of each month and submit to Microbiologist for review.
- 16) Record all data in ink with changes lined through such that original entry is visible. Changes are initialed and dated

3.3. Principal Analyst Personnel Qualifications

3.3.1. Principal Analyst Personnel Qualifications in QA Manual

3.3.2. Personnel Training and Competency Record

Section: Water Laboratory

Period _____ to _____

Employee Name: _____

Procedure	Training			Competency		
	Review Date	Employee Initials	Supv. Initials	Procedure Type*	Date Reviewed	Supv Initials
Monitoring equipment temperature						
Sample receiving						
Setting up/labeling media for MF						
Perform Membrane Filtration						
Setting up Precision Criteria						
Reading Les Endo						
Reading mFC						
Reading mEI						
Reading mTEC						
Calculate MF results						
Sign out MF membrane sterility QC						
Sign out lab assistant daily method precision						
E-mailing reports						
Faxing reports						
Mailing reports						
Signing out water reports						
Perform Data Entry						
Discarding water samples						
Filing water report forms						
Writing requisitions						
Filing requisitions						
Performing inventory						
Entering monthly tally reports						
Harvesting QC organisms						
Setting up Quality Control						
Reading/recording QC						
Reading HPC for Milli-Q water						
Signing out QC for Milli-Q water						
Freezing cultures						
Multiple Tube Fermentation (MTF)						
Setting up Colilert 18/Enterolert						
Reading Colilert 18/Enterolert						
Reporting positive domestics						
ELAP proficiency samples						

*Procedure type; Personal Observation (PO), Written exam (EX), Feedback (FB), Review of Record (ROR), Review of documentation (ROD)

4. Sample Collection, Transport and Disposal

4.1. Water Sample Collection SOP

1) SAMPLE CONTAINERS

- a) For bacteriological samples, use sterilized bottles of glass or plastic of appropriate size and shape. Bottles must be capable of holding a sufficient volume of sample for all required tests, while allowing for air space. Wide-mouthed autoclavable or pre-sterilized Polypropylene bottles of suitable size commercially available are satisfactory. Pre-sterilized plastic bags, with or without a de-chlorinating agent, may also be used. Containers for collecting water may have residual chlorine or another halogen and therefore should contain a reducing agent such as sodium thiosulfate. Sodium thiosulfate neutralizes any residual halogen and prevents the continuation of bactericidal action during sample transit.
- b) OCHD water collection bottles are quality controlled by batch (See Media QC Notebook). Sample bottles are stored in the water laboratory for use by Environmental Health personnel and other governmental agencies upon request.

2) SAMPLE COLLECTION

- a) When collecting the sample, leave enough air space in the bottle to allow for proper mixing before examination. Collect samples that are representative of the water being tested. Flush or disinfect sample ports, and use aseptic techniques to avoid sample contamination. Keep the sample bottle closed until it is to be filled. Remove cap carefully to avoid contaminating the inner surface of the cap and neck of the bottle. Fill container without rinsing. Replace cap immediately. The volume of sample should be sufficient to carry out all tests required (not less than 100 ml). Provide complete and accurate sample identification and description information as specified on the sample collection sheet (chain of custody form).

3) SAMPLE TYPES

- a) Potable Water
 - i) For drinking water analysis, collect samples consisting of finished water.
 - ii) Open tap fully and flush water for 2 - 3 minutes, or until a time sufficient to permit clearing the service line.
 - iii) Reduce water flow to permit filling bottle without splashing.
 - iv) Do not sample from leaking taps that allow water to flow over the outside of the tap.
 - v) When sampling from a mixing faucet remove faucet attachments, run hot water for 2 minutes, then cold water for 2 to 3 minutes, and collect sample as indicated above.
 - vi) If the sample is taken from a well fitted with a hand pump, pump water for 5 minutes before collecting sample.
 - vii) If the well is equipped with a mechanical pump, collect sample from a tap on the discharge.
 - viii) If there is no pumping machinery, collect a sample directly from the well.
- b) Raw water:
 - i) For collecting samples directly from a river, stream, lake, reservoir, spring, or shallow well, obtain samples representative of the water that is the source of supply to consumers.
- c) Surface waters:
 - i) Select sampling locations to include a baseline location upstream from the study area. Where a tributary stream is involved, select the sampling point near

the confluence with the mainstream points. To monitor stream and lake water quality, establish sampling locations at critical sites. Sampling frequency may be seasonal.

d) Bathing beaches:

- i) Sampling locations for recreational areas should reflect water quality within the entire recreational zone. Collect samples in the swimming area at ankle depth. As much as possible, avoid collecting sand, debris or sand crabs that may be suspended in the water. To avoid self-contamination of sample, use a sampling pole that is designed to hold a sample bottle. Collect samples on an incoming wave. To obtain baseline data on marine and estuarine bathing water quality include sampling at low, high, and ebb tides. Relate sampling frequency directly to the peak bathing period.

e) Swimming pools:

- i) A swimming pool is a body of water of limited size contained in a holding structure. The water is generally chlorinated potable water but may also be derived from thermal springs or saltwater. Collect samples in the area and time of maximum bather density. Collect samples where water is at least 1 meter deep. (See Std. Methods 17th Edition Section 9213 B. for further information.)

f) Sediment and sludge:

- i) Sediments provide a stable index of the general quality of the overlying water. Sampling frequency may be related to seasonal changes in water temperatures and storm water runoff. Sludge monitoring may indicate the effectiveness of wastewater treatment processes. Bottom sediment sampling requires special apparatus. (See Std. Methods 9060 A. 3. g.)

4) HOLDING TIME AND TEMPERATURE

a) General:

- i) Start microbial analysis of water samples as soon as possible after collection to avoid unpredictable changes in the microbial population. Keep samples cold during transport to the laboratory (4-10°C), if they cannot be processed within 1 hour after collection. Blue ice packs are preferred over ice. If using ice, avoid direct contact of samples with ice using plastic packing material.

b) Drinking Water for Compliance purposes:

- i) Hold samples at 4-10°C during transit to the laboratory. Analyze samples on day of receipt whenever possible and refrigerate overnight if arrival is too late for processing on same day. Do not exceed 30-hour holding time from collection to analysis for coliform bacteria. Do not exceed 8 hour holding time for heterotrophic plate counts.

c) Non-potable Water for Compliance purposes:

- i) Hold source water, stream pollution, recreational water, and wastewater samples between 4-10°C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours. When transport of samples is longer than 6 hours consider using delayed incubation procedures.

d) Water for Non-compliance purposes:

- i) Hold samples between 4-10°C during transport and until time of analysis. Do not exceed 24-hour holding time.

4.2. Sample Collection Bottle QC Form (sample)

Product: Collection Bottle QC Organisms: Sterility only Incubation Requirements: 35°C, O ₂ , 48 Hrs. Test 5%, or 5 bottles from each box, for sterility. Add 25ml Nutrient Broth to each bottle.
--

Date in QC	Date out QC	Lot #	Exp. Date	Sterile	Initial	Comments

4.3. Sample Receiving SOP

- 1) Use proper safety precautions when handling water samples.
- 2) Check the paperwork to make sure the right form is being used and that all the necessary information is filled in correctly:
 - a) Date collected
 - b) Time collected: Samples should arrive to the lab within 6hrs after being collected.
 - c) Last name of sampler.
 - d) Type of water sample (i.e. marine, domestic, sewage, groundwater, surface water, reclaimed).
 - e) Location and station number.
 - f) Test requested.
- 3) Along with sampler, read and record temperature on the water lab sign in sheet along with date received, the time the samples arrived and initials.
 - a) NOTE: Temperature should be greater than 0°C and less than 10° C.
- 4) Verify test and dilutions needed with the “Summary of water sample dilution chart”.
- 5) Using the pre-printed labels, number each sample (on COC and sample bottle) with a WL number consecutively maintaining the numbered order set-up by the sampler. Check the last WL number from previous day before numbering. DO NOT SKIP ANY NUMBERS!
- 6) Indicate time samples received on the water lab reports.
- 7) Indicate the time samples run on the water lab reports and initial.
- 8) Compliance samples should be processed within 6 hours from collection time. Non-Compliance samples should be processed no later than 24 hours from time of collection

4.4. Laboratory Sign-in Sheet (sample)

DATE	TIME IN	AMOUNT OF SAMPLES		TEMPERATURE <10°C	INITIALS	COMMENTS
		RECREATIONAL	DOMESTIC			

4.5. Water Sample Disposal Procedure

- 1) Sewage Samples
 - a) All samples associated with sewage or a sewage spill must be disposed of in the following manner.
 - b) Process samples as stated in the SOP for specific requests.
 - c) After processing, replace the lid to the collection bottle.
 - d) Dispose sample and collection bottle into a red biohazard bag to be autoclaved.
- 2) Recreational/Domestic Samples
 - a) Process samples as stated in the SOP for specific test requests.
 - b) After processing, discard sample, opened collection-bottle, and lid into a tub half filled with a 10% bleach solution. Always wear protective equipment when working with bleach (i.e. latex gloves and eye protection)
 - c) Preparation of 10% bleach solution
 - i) Fill a 2 gallon Nalgene bottle half full with tap water
 - ii) Add 800ml of bleach into the half full Nalgene bottle
 - iii) Fill the remainder of the bottle with tap water leaving about 3 inches of air space from the lid.
 - iv) Close the Nalgene bottle firmly and invert to mix the solution evenly.
 - d) Allow sample and collection bottle to soak in bleach solution for a minimum of 30 minutes.
 - e) After the 30-minute soak, remove the collection-bottles and lids and discard them into regular trash.
 - f) With liberal amounts of cold water discard the bleach solution down the sink drain, careful to avoid splashing.
 - g) Allow the cold tap water to run for 2 minutes after discarding bleach.
- 3) Sediment Samples
 - a) Process samples as stated in the SOP for specific requests.
 - b) After processing, add 10% bleach to the sediment bottles and re-cap. Always wear protective equipment when working with bleach (Latex gloves and eye protection).
 - c) Preparation of a 10% bleach solution (see step 2.1 above).
 - d) Allow sample and collection bottle to soak in bleach solution for a minimum of 30 minutes.
 - e) Dispose of collection bottle into regular trash.

5. Data Reporting

5.1. Reporting Test Results SOP

- 1) Microbiologists/Lab Assistants input the results into the CERNER PathNet system on the day that membrane filtration plates are read.
- 2) All results will be reviewed for data entry accuracy prior to verification of calculated results.
- 3) Any faxed, e-mailed, or verbal notification results will be documented on the COC in the comments field.
 - a) For electronic submission of results refer to the Data Handling Procedure
- 4) Follow the instructions listed below to report data for each submitter:

Submittor	Study	Regular Collection Day	Regular Sampler	COC to:	Report to:
OC Water District (OCWD)	SARMON			OCWD	OCWD
	GWRS			OCWD	
Camp, Dresser, McKee, Smith (CDM Smith)	MSAR	Tues		CDM	CDM
Env Health (EH)	RT - Seal Beach/Huntington Harbor	Thurs	Halle	EH	EH
	RT - Newport Bay	Mon	Halle	EH	EH
	RT - Newport Coast	Thurs	Halle	EH	EH
	RT - Laguna Beach	Tues	Halle	EH	EH
	RT - Dana Point Harbor	Tues	Anzora	EH	EH and OCPW
	AB411 / Sewage Spill			EH	EH
	RT - Domestics (Public Utilities / USDA)		Anzora	EH	EH - Call any positives to Larry Brenner
OC Public Works (OCPW)	SDR CSDO - Laguna Bch	Tues	Halle	OCPW and EH	OCPW and EH
	SDR CSDO - San Clemente	Wed	Halle	OCPW and EH	OCPW and EH
	SJC TMDL Pathogen				OCPW
	SAR Pathogen - Costa Mesa/Bolsa Chica	Thurs	Halle		OCPW
	BMP Coastal Stormdrain Maintenance				OCPW
	ALC Aliso Creek				OCPW
	SDR Mass Emmissions				OCPW
	SAR DWM Dry Weather Monitoirng				OCPW
	SDR DWM Dry Weather Monitoring	Mon - Thur			OCPW

Instructions for sending COC's

OCWD - each day sampler drops off samples make a copy of the COC's and give to sampler before they leave. Batch the completed COC's in the black file organizer and scan and email to Nira Yamachika (Nyamachika@ocwd.com) at the end of each month. Send each day's COC's as separate emails to yourself then forward from your Inbox to Nira. File the COC's in the monthly folder by date.

CDM - Collect all completed COC's in the black file organizer. At the end of the month scan and email the COC's to Tiffany Lin (lnty@cdmsmith.com). Send each day's COC's as separate emails to yourself then forward from your Inbox to Tiffany. File the COC's in the monthly folder by date.

EH - Tami will make a copy of the COC's after we log them in. If Tami is not the sampler, then make copies and instruct sampler to give copies to Tami.

OCPW and EH - Tami will make a copy of the COC's. Scan and email COC's to yourself. Forward email from your Inbox to Rita Abellar (rita.abellar@ocpw.ocgov.com). Use the stamper to place a blue check mark on the upper right hand corner of the COC's to indicate that the COC's were sent.

Instructions for reporting results

OCWD - Do not verify results with ">" qualifier. Give to Joe to verify. Results without qualifiers can be verified, email results spreadsheet to Nira Yamachika (NYamachika@ocwd.com). Patrick Versluis (PVersluis@ocwd.com) and Rita Hintlian (RHintlian@ocwd.com)

CDM - As each day's results are verified, email results spreadsheet to Tiffany Lin (lnty@cdmsmith.com). As TSS results are reported by Manisha generate results spreadsheet and email to Tiffany Lin.

EH - As each day's results are verified, email results spreadsheet to Larry Brennler (lbrennler@ochca.com), Dan Yokoyama (dyokoyama@ochca.com), Juan Anzora (janzora@ochca.com), Lauren Hatch (lhatch@ochca.com), Kristen Schroeder (kschroeder@ochca.com), and Tami Halle (thalle@ochca.com)

OCPW - As each day's results are verified, email results spreadsheet to Rita Abellar (rita.abellar@ocpw.ocgov.com), Kacen Clapper (kacen.clapper@ocpw.ocgov.com), Jonathan Curry (jonathan.curry@ocpw.ocgov.com), Mike Fennessy (michael.fennessy@ocpw.ocgov.com), James Fortuna (james.fortuna@ocpw.ocgov.com), Suzan Given (suzan.given@ocpw.ocgov.com), Jon Lewengrub (jon.lewengrub@ocpw.ocgov.com), Joel Magsalin (joel.magsalin@ocpw.ocgov.com), Ann Mesa (ann.mesa@ocpw.ocgov.com), Leonard Narel (leonard.narel@ocpw.ocgov.com), Robert Rodarte (robert.rodarte@ocpw.ocgov.com)

Create distribution lists for **EH** and **OCPW** to make it easier for reporting. **Make sure to send verified results to both submitters when indicated.**

Abbreviations: SARMON - Santa Ana River Monitoring, GWRS - Ground Water Replenishment System, MSAR - Middle Santa Ana River, RT - Routine, AB411 - Assembly Bill 411, SAR - Santa Ana Region, SDR - San Diego Region, CSDO - Coastal Stormdrain Outfall, SJC - San Juan Creek, TMDL - Total Maximum Daily Load, BMP - Best Management Practice, ALC - Aliso Creek, DWM - Dry Weather Monitoring

5.2. Data Handling Procedure

1) Purpose:

- a) The purpose of this procedure is to outline the steps required to properly document and report data results. The Water Quality Laboratory submits a majority of its results via e-mail and specific steps are required to make sure every result is reported accurately.


2) Procedure:

- a) Receive and process samples according to SOP.
- b) Initial Data Entry
 - i) Initial data entry will be performed the same day samples are processed, and/or the following day, before sample results are read and recorded.
 - ii) The lab uses Cerner Millenium for its lab information system (LIS)
 - iii) Use the COC to register samples and order tests in the LIS using the Department Order Entry (DOE) application
 - iv) After registration use the Specimen Log-in application to indicate that the specimens are in the lab and ready to be resultated.

- c) Result Entry
 - i) Use the Accession Result Entry (ARE) application to enter results
 - ii) Use the assigned accession number to bring up the sample to be result.
 - iii) Enter all sample volumes tested and the counts for each plate and for each media set up.
 - iv) After all counts are entered for the sample click on PERFORM. NOTE: you must click on PERFORM and not VERIFY in order for the results to get calculated. If VERIFY is clicked in error prior to PERFORM consult with the Micro II or Supervisor to get calculated results entered.
 - v) Enter the next accession number to be result. Continue reading, entering counts, and PERFORMing results for the remainder of samples.
- d) Calculation verification
 - i) Once all counts have been entered and PERFORM'd, the calculated results will need to be verified.
 - ii) Use the ARE application to verify the results.
 - iii) Use the assigned accession number to bring up the sample results that need to be verified.
 - iv) Double check all previously entered sample volumes and counts for accuracy then check that the calculated results are correct. If errors in sample volumes or counts were detected and corrected data was entered, then results must be re-PERFORM'd before verifying. If no corrections were needed then click on VERIFY to make results available for reporting.
 - v) Enter the next accession number for results to be verified. Continue checking the accuracy of the data entered, and the accuracy of the calculated result, and VERIFY results for the remainder of samples.
 - vi) All OCWD results shall be PERFORM'd and VERIFY'd only if there are no "≥" qualifiers. For OCWD samples with the "≥" qualifier, leave the sample in the PERFORM'd status and the Supervisor will review and VERIFY the results.
- e) Export Data
 - i) Once the results have been double checked and verified, use the Explorer Menu application to export the data from Cerner into an Excel spreadsheet.
 - ii) Select the WQL report in the Explorer Menu application to generate the spreadsheet for each client and each day's results.
 - iii) Save the exported spreadsheets in each client's folder in the Exported xls file on the PHwaterLab share folder.
 - iv) Save exported data reports
 - (1) Add the date of sample collection to the file name and include initials of staff creating the export file.
 - (2) For example an EH export file emailed on 1-05-09 containing information on samples collected on 1-04-09 will be named: "1-04-09 JG.xls"
 - v) Place the file into a folder within the share folder with corresponding dates (mm-yy)

- f) Proofread Data
 - i) After exporting and saving the results spreadsheet into the appropriate folder, open up the file for proofreading before emailing to client.
 - ii) Review the file for skips in accession numbers or multiple study names for the same sample. Also review for unusual qualifiers, such as “TNTC” or for unusual numeric results, such as “0.00”. Make corrections to the spreadsheet as needed.
- g) E-mail Data
 - i) Attach the file to an e-mail and send it to the submitting agency
 - ii) Follow emailing instruction described in the Data Reporting section.

5.3. Membrane Filtration Report Form (blank)

		County of Orange, Health Care Agency Water Quality Laboratory (ELAP # 2545) 600 Shellmaker Rd. Bldg. A Newport Beach, CA 92660 Phone: (949)219-0423 FAX: (949)219-0426		Client:		
				Study/Billing Code:		
				Contact Info:		
				Date Collected:		
				Sampler Name:		
Bottle #	Time Collected	MRN/Station ID, Location	Sampler Comments	Submitter Accession #	Lab Accession #	Test Requested
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
Name / Date / Time		Comments:				
Relinquished by:						
Received by:						
Analyzed by:						
Reviewed by:						
Reported to:		Transport Conditions:				


5.4. Membrane Filtration Report Form (sample)

		County of Orange, Health Care Agency Water Quality Laboratory (ELAP # 2545) 600 Shellmaker Rd. Bldg. A Newport Beach, CA 92660 Phone: (949)219-0423 FAX: (949)219-0426		Client: RDMD		
				Study/Billing Code: Aliso Creek / EV85007		
				Contact Info: Rita.Abellar@rdmd.ocgov.com		
				Date Collected: 6/19/2008		
				Sampler Name: Simionescu, Gheorghe		
Bottle #	Time Collected	MRN/Station ID, Location	Sampler Comments	Submitter Accession #	Lab Accession #	Test Requested
1	7:13	MRN: 1000217112  PHEH Water Quality Station ID: BDP16 Location: PILGRIM	5 birds 10 school children overboard	WR0012345		<input checked="" type="checkbox"/> Total Coliform <input checked="" type="checkbox"/> Fecal Coliform <input checked="" type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input checked="" type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input checked="" type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Water Type: <input type="checkbox"/> Domestic <input type="checkbox"/> Surface <input type="checkbox"/> Marine <input type="checkbox"/> Ground <input type="checkbox"/> Reclaimed <input type="checkbox"/> Other		Preservative: <input type="checkbox"/> Na ₂ S ₂ O ₃ <input type="checkbox"/> None <input type="checkbox"/> Other				
						<input type="checkbox"/> Total Coliform <input type="checkbox"/> Fecal Coliform <input type="checkbox"/> Enterococci <input type="checkbox"/> E. coli
Name / Date / Time				Comments: Transport Conditions:		
Relinquished by:						
Received by:						
Analyzed by:						
Reviewed by:						
Reported to:						

5.5. Colilert™/Multi-Tube Fermentation Report Form (blank)

		County of Orange, Health Care Agency Water Quality Laboratory 700 Shellmaker Rd. Newport Beach, CA 92660 ELAP #2545 PHONE: (949) 219-0423 FAX: (949) 219-0426				LOCATION: _____ SAMPLE TYPE: <input type="checkbox"/> Recreational <input type="checkbox"/> Domestic <input type="checkbox"/> Other _____ WEATHER: _____						
FIELD DATA		LABORATORY REPORT										
Date Collected _____ By _____		Total Dilutions # _____ Samples # _____		Date Received _____ Received By _____ Time In _____ Time Run _____ Date Read/By _____ Time C-18 Read _____ Time ENT Read _____								
Field Bottle #	Time Clz/room	Site No. / Location of Sampling Station		Dilution Used	Yellow Color at 18hrs	Total Coliform MPN/100ml	Fluorescence at 18hrs	E. coli MPN/100ml	Dilution Used	Blue Fluores. at 24hrs	Enterococci MPN/100ml	Report Date/By
<input type="checkbox"/> Panel (QT, Entero, MTF) <input type="checkbox"/> Colilert <input type="checkbox"/> Enterolert <input type="checkbox"/> MTF Inc. Dil _____			0		Lg wells Sm wells		Lg wells Sm wells			Lg wells Sm wells		
			1									
			2	Vol. ml	Presump.	Confirmation	F.C.	Total Coliform MPN/100ml	Fecal Coliform MPN/100ml			
			3	1.0								
			4	0.1								
			0.01									
			0.001									
			0.0001									
			0		Lg wells Sm wells		Lg wells Sm wells			Lg wells Sm wells		
			1									
			2	Vol. ml	Presump.	Confirmation	F.C.	Total Coliform MPN/100ml	Fecal Coliform MPN/100ml			
			3	1.0								
			4	0.1								
			0.01									
			0.001									
			0.0001									
			0		Lg wells Sm wells		Lg wells Sm wells			Lg wells Sm wells		
			1									
			2	Vol. ml	Presump.	Confirmation	F.C.	Total Coliform MPN/100ml	Fecal Coliform MPN/100ml			
			3	1.0								
			4	0.1								
			0.01									
			0.001									
			0.0001									
			0		Lg wells Sm wells		Lg wells Sm wells			Lg wells Sm wells		
			1									
			2	Vol. ml	Presump.	Confirmation	F.C.	Total Coliform MPN/100ml	Fecal Coliform MPN/100ml			
			3	1.0								
			4	0.1								
			0.01									
			0.001									
			0.0001									
			0		Lg wells Sm wells		Lg wells Sm wells			Lg wells Sm wells		
			1									
			2	Vol. ml	Presump.	Confirmation	F.C.	Total Coliform MPN/100ml	Fecal Coliform MPN/100ml			
			3	1.0								
			4	0.1								
			0.01									
			0.001									
			0.0001									
SUBMITTOR INFORMATION / SUBMITTOR NUMBER _____ Orange County Health Care Agency/Environmental Health 2009 East Edinger, Santa Ana, CA 92705 PHONE: (714) 667-3600 FAX: (714) 972-0749				Field or Lab Remarks: _____								

5.6. Heterotrophic Plate Count Report Form (blank)

		County of Orange, Health Care Agency Water Quality Laboratory 700 Shellmaker Rd. Newport Beach, CA 92660 ELAP #2545 PHONE: (949) 219-0423 FAX: (949) 219-0426		LOCATION: _____					
				SAMPLE TYPE: <input type="checkbox"/> Recreational <input type="checkbox"/> Domestic <input type="checkbox"/> Other _____					
WEATHER: _____									
FIELD DATA			LABORATORY REPORT						
Date Collected _____ By _____		Total Dilution 1: _____ 2: _____ 3: _____ 4: _____	Date Received _____ Time In _____		Received By _____ Time Run _____				
Field Bottle #	Time Clz/ppm		Sln. No. / Location of Sampling Station						
			Sample Used	Colony count Plate #1(P1)	Colony count Plate #2(P2)	Average(0.1) (P1+P2)/2	Average(0.1) x 10	CFU/ml Ave. (0.1) + Ave. (1.0) / 2	Report Date/By
			0.1						
			Sample Used	Colony count Plate #3(P3)	Colony count Plate #4(P4)	Average(1.0) (P3+P4)/2			
			1.0						
<input type="checkbox"/> Heterotrophic Plate Count		Laboratory No. _____							

5.7. Result Notification Log Form (sample)

*Record inclusive lab numbers in the corresponding columns

DATE	TIME		INDIVIDUAL CONTACTED	MF	DOMESTIC	INITIAL
	FAX	CALL		FINAL REPORTS	COLILERT	

5.8. Laboratory Disposal of Records Memo



COUNTY OF ORANGE HEALTH CARE AGENCY

PUBLIC HEALTH LABORATORY WATER QUALITY

MARK REFOWITZ
DIRECTOR

RICHARD SANCHEZ
ASSISTANT DIRECTOR

ERIC G. HANDLER, MD, MPH, FAAP
Deputy Agency Director/Health Officer

RICHARD ALEXANDER, MS, MPH
Laboratory Director

MAILING ADDRESS:
600 SHELLMAKER RD., BLDG A
NEWPORT BEACH, CA 92260

PHONE: (949) 219-0423
FAX: (949) 219-0426
E-MAIL: RAlexander@ochca.com

DATE: January 28, 2015
TO: HCA Water Quality Laboratory Submitters
FROM: Joseph A. Guzman, Water Lab Supervisor
SUBJECT: Disposal of 2008 laboratory records

The 22nd Edition of Standard Methods for the Examination of Water and Wastewater Part 9020B Section 12-c states that laboratories shall keep records of microbial analysis for at least 5 years in a secure location. This communication will serve as official notification to HCA Water Quality Laboratory Submitters that laboratory records prior to, and up to December 31, 2008, regarding sample analysis and quality control, will be disposed of on March 1, 2015. If your program has a need for these older records or has questions regarding these records, please contact the laboratory prior to March 1, 2015.

Thank you,

Joseph A. Guzman
Supervising Public Health Microbiologist
Orange County Public Health Water Quality Laboratory
600 Shellmaker Rd., Bldg A, Newport Beach, CA 92660
Direct: 949-219-0424 Fax: 949-219-0426



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6. Laboratory Equipment

6.1. Equipment QA Summary

Maintaining proper temperatures in the Water Lab is of utmost importance. Forma O₂ incubator temperatures are checked twice daily using calibrated thermometers on each shelf. The water bath is also checked twice daily, at least four hours apart. Freezers and refrigerators are checked once a day. Temperatures are recorded on a temperature chart specific for each unit. Any temperatures out of range are noted in red and a Microbiologist is notified to take immediate corrective action. All corrective actions are noted on the temperature chart in its appropriate column. All of the equipment is kept on a monthly primary maintenance schedule contracted out to a private maintenance company. **Thermometers are calibrated yearly, and all calibration reports are kept in the Water Quality Laboratory.** Laboratory Assistants clean each unit monthly to maintain a clean working environment free of contaminants.

6.2. Laboratory Equipment List

Equipment	Manufacturer	Model	S/N	FA #	CE #	Maintenance Schedule	Date Last Calibration
Autoclave SMALL UNIT PREVACCUUM #1	Steris	SV-120	0123105-08	61771	N/A	Quarterly	3/18/2014
Balance	Mettler Toledo	PN1210	648001	N/A	3606	N/A	5/9/2014
Balance	Denver Instrument	APX-200	A24062012	N/A	14610	N/A	5/9/2014
Colony Counter	Quebec	3325	3885113	N/A	29503	N/A	N/A
Conductivity Meter	Mettler Toledo	S30	122847524 1	N/A	22428	N/A	Calibrated as Needed
Conductivity Meter	Thermo Orion	115A Plus	004353	N/A	N/A	N/A	Calibrated as Needed
Freezer #1	Sanyo	MDF-U73VC	09010051	73757	N/A	Monthly	2/27/2014
Freezer #3	Sanyo	MDF-U72VC	31011157	61661	N/A	Monthly	2/27/2014
Incubator #1	Forma Scientific	3960	92364-48	61463	N/A	Quarterly	3/19/2014
Incubator #2	Forma Scientific	3960	97211-19	61426	N/A	Quarterly	3/19/2014
Incubator #5	Millipore	XX632000	4689	N/A	14184	Quarterly	3/19/2014
Incubator #6	Millipore	XX632000	4827	N/A	14611	Quarterly	3/19/2014
Incubator #7	Millipore	XX632000	4829	N/A	14612	Quarterly	3/19/2014
Incubator #8	ThermoForma	3911	300352-152	61522	N/A	Quarterly	3/19/2014
Incubator #9	ThermoForma	3911	300352-153	61523	N/A	Quarterly	3/19/2014
Incubator #10	Sanyo	MIR-153	31011190	N/A	17100	Quarterly	3/19/2014
pH Meter	Corning	240	6260	N/A	N/A	Quarterly	Calibrated as Needed

Refrigerator #4	Sanyo	MPR-1410	20100453	N/A	14782	Monthly	2/27/2014
Refrigerator #5 (w/freezer)	Sanyo	MPR-414F	31213626	N/A	17101	Monthly	2/27/2014
Refrigerator #6	Sanyo	MPR-1410	31213665	N/A	16169	Monthly	2/27/2014
Refrigerator #7	Sanyo	MPR-1410	41014666	N/A	18572	Monthly	2/27/2014
Refrigerator #8	Sanyo	MPR-1410	41013398	N/A	18573	Monthly	2/27/2014
Refrigerator #9	Sanyo	MPR-513	41014189	N/A	18544	Monthly	2/27/2014
Refrigerator #10	Sanyo	MPR-1410	61119064	72004	N/A	Monthly	2/27/2014
UV Light (254 & 366 nm)	CAMAG	CAMAG	730806	N/A	N/A	Monthly	N/A
Waterbath #2	PolyScience	8306A11B	1A1240121	TBA	N/A	Monthly	3/18/2014
Beadbath	LabArmor	3325	10501-9	TBA	N/A	Monthly	Mar-14
MICROSCOPE	NIKON	LABOPHOT	242129/232 932	3717	636	Semi-Annual	4/29/2014
MICROSCOPE	NIKON	LABOPHOT	218065		7722	Semi-Annual	4/29/2014
MICROSCOPE	NIKON	SMZ645	1026774		12582	Semi-Annual	4/29/2014
MICROSCOPE	NIKON	SMZ645	1026523		12583	Semi-Annual	4/29/2014
MICROSCOPE	NIKON	SMZ645	1064654		18502	Semi-Annual	4/29/2014
MICROSCOPE	NIKON	SMZ745	1095499		29505	Semi-Annual	4/29/2014
QUANTI-TRAY SEALER	IDEXX	89-10894-00	1462			Monthly	6/30/2014
MEDIA ROOM							
Autoclave #1	Steris	Amisco Century SV- 120 Scientific P revac	0123105-08		61773	Quarterly	4/2/2014
Autoclave #2	Steris	Amisco Century SV- 120 Scientific P revac	0122905-03		61772	Quarterly	4/2/2014
Autoclave #3	Steris	Amisco Century SV- 120 Scientific P revac	0122805-12		61773	Quarterly	4/2/2014
Balance Mettler PB3002-S/Facti	Mettler Toledo Instrument	PB3002-S	112901256 9		22426	Semi-Annual	5/14/2014
Balance Mettler Toledo PG503-S	Monobloc Comp	PG503-S	112049091 8		15454	Semi-Annual	5/14/2014
Weights (for Balances)	Mettler Toledo / Troemner Weight set	PG-503-S	31590	22429		Every 5 yrs	5/20/2009
Oven, Gravity Convection	Lindberg	G01390A	Y26L55752 3-2Z		13440	Yearly	3/19/2014
Ph Meter Mettler Toledo SevenEasy	Mettler ToledoGmbH	S20	122835563 3			Semi-Annual	3/17/2014
Orion Star pH Meter A111	Thermo Scientific	S20	J04744	29015	N/A	Semi-Annual	3/17/2014

Conductivity Meter	Mettler Toledo GmbH Analytical	G01390	122847524		22425	Semi-Annual	3/17/2014
Refrigerate/Freezer Sanyo	Sonyo Scientific	MPR-411F	1111011		12157	Semi-Annual	3/17/2014
Water Bath	Labcare America	266	601091683		13441	Semi-Annual	3/17/2014
Automatic Pipeting Machine	Sepco	40A	1042			Semi-Annual	3/17/2014
Automatic Pipeting Machine	Sepco	40A	1125			Semi-Annual	3/17/2014
Automatic Pipeting Machine(Oxford)	Oxford Labware	8885-047004(115V)	5855			Semi-Annual	3/17/2014
Automatic Pipeting Machine(Oxford)	Oxford Labware	8885-047004(115V)	10112363			Semi-Annual	3/17/2014
Miele Large Dishwasher	Miele	G7735MCU	000/53076190		61462	Semi-Annual	2/10/2014
Miele Small Dishwasher	Miele	G7735CD	53084488		61524	Semi-Annual	2/10/2014

6.3. Equipment Maintenance/Repair Log form (Blank)

Item: Model #: Fixed Asset #: County #: Serial #:				
DATE NOTED	PROBLEM NOTICED	REPAIRS PERFORMED	DATE FIXED	INITIALS

6.4. Procedure for Checking Temperatures on Equipment

- 1) Temperatures are recorded on each piece of equipment daily in the section by the Laboratory Assistants in accordance with the following procedure.
 - a) Read and record temperature based on the calibrated thermometers on all incubators, refrigerators and water baths. Waterlab freezers have external thermometers and do not require separate internal readings.
 - b) Note:
 - i) Thermometers should be enclosed in water to prevent major fluctuation in temperature during reading.
 - ii) All correction factors, which are written on the calibration sticker, must be taken into consideration when reading the temperature.
 - iii) There should be no separation in the fluid column of the thermometer. Notify a Microbiologist immediately.
 - iv) Thermometers discarded if off more than 1°C from reference thermometer.
 - c) Record the temperature, in black ink, to the nearest 0.1 degree for the incubators and water baths, and to the nearest 1 degree for the refrigerators. The QC charts are located on each instrument.
 - d) If any equipment is outside the specified temperature range (the range is stated on the top of every QC chart), record the temperature in red ink and notify a Microbiologist immediately for corrective action.
 - i) The Microbiologist making the corrective action will re-check the parameters before making any adjustments to the equipment (i.e. possible reading errors or defective monitoring device).
 - ii) The Microbiologist will make all minor adjustments to the equipment operation manuals (kept in the equipment folder) and record the corrective action on the QC chart.
 - iii) The equipment is read again in a reasonable amount of time to ensure that the equipment is within range.
 - iv) If the corrective action does not resolve the problem, a request for equipment repair is issued according to the following procedure:
 - (1) Microbiologist will locate the folder for equipment needing repair and/or service.
 - (2) Indicate date, describe problem on equipment log sheet and notify office in main lab. (Attach a note to folder, contact micro name). Office will call in repair to purchasing and keep folder in office.

Repairman will arrive to the lab. Micro/Supervisor will indicate repairs performed on the equipment log, date and initial equipment log sheet in the appropriate column. Any required signatures are to be signed by Supervisor/Micro.

- (3) Place copy of invoice in equipment log and send original to HCA Purchasing

Temperature	Thermometer Increments
44.5°C ± 0.2°C	≤ 0.2°C
35.0°C ± 0.5°C	≤ 0.5°C
~5°C (refrigerators)	≤ 1.0°C

6.5. Monthly Incubator Temperature Log (Blank)

ORANGE COUNTY
LABORATORY

WATER LAB TEMPERATURE RECORD CHART

EQUIPMENT: INCUBATOR #

FA #

CO: # N/A

SN: #

W=WEEKEND

H=HOLIDAY

MONTH	SHELF 1		SHELF 2		SHELF 3		SHELF 4		SHELF 5		SHELF 6	
Feb-03	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1-W												
2-W												
3												
4												
5												
6												
7												
8-W												
9-W												
10												
11												
12-H												
13												
14												
15-W												
16-W												
17-H												
18												
19												
20												
21												
22-W												
23-W												
24												
25												
26												
27												
28												

6.6. Monthly Waterbath Temperature Log (Blank)

**ORANGE COUNTY
LABORATORY**

W=WEEKEND

H=HOLIDAY

WATER LAB TEMPERATURE RECORD CHART

EQUIPMENT: WATERBATH #

TEMP RANGE: 44.5°C±0.2°C

FA: #

CO: # NA

SN: #

Feb-03				CORRECTIVE ACTIONS
DAY	AM	PM	INITIAL	
1-H				
2				
3				
4-W				
5-W				
6				
7				
8				
9				
10				
11-W				
12-W				
13				
14				
15				
16				
17				
18-W				
19-W				
20-H				
21				
22				
23				
24				
25-W				
26-W				
27				
28				
29				
30				
31				
SUPERVISOR REVIEW				

6.7. Monthly Refrigerator Temperature Log (Blank)

**ORANGE COUNTY
LABORATORY**

**W=WEEKEND
H=HOLIDAY**

WATER LAB TEMPERATURE RECORD CHART

EQUIPMENT: REFRIGERATOR #
TEMP RANGE: REF 5±3°C; FREEZER -15±5°C

FA: # NA SN: #
CO: # NA

Sep-03				CORRECTIVE ACTIONS
DAY	REF	FREEZER	INITIAL	
1 H				
2				
3				
4				
5				
6 W				
7 W				
8				
9				
10				
11				
12				
13 W				
14 W				
15				
16				
17				
18				
19				
20 W				
21 W				
22				
23				
24				
25				
26				
27 W				
28 W				
29				
30				
SUPERVISOR REVIEW				

6.8. List of Certified Thermometers

THERMOMETER - SERIAL# /LAB#	MANUFACTURER	TYPE (spirit-filled, mercury, digital)	RANGE	DEPT	LOCATION / STATUS	EQUIPMENT (INCUBATOR, waterbath, refrig.)	BI-ANNUAL CALIBRATION 2013-2014	CALIBRATION DUE DATE	CORRECTION FACTOR	COMMENTS
518	VWR SCIENTIFIC	MERCURY	35C/46C	WL	WL	WATER BATH #2	9/9/2014	3/9/2015	0.20	IN USE
947	ERTCO	SPIRIT-FILLED	-50C/50C	WL	WL	REFRIGERATOR #9	9/9/2014	3/9/2015	-0.20	IN USE
1029	ERTCO	SPIRIT-FILLED	-50C/50C	WL	WL	REF./FREEZER #5	9/9/2014	3/9/2015	0.40	IN USE
1105	A&M THER. CORP.	SPIRIT-FILLED	20C/110C	WL	WL	BEAD BATH	9/9/2014	3/9/2015	0.50	IN USE
1808	ERTCO	MERCURY	34.5C/46.5 C	WL	WL	INCUBATOR #8	9/9/2014	3/9/2015	0.30	IN USE
1809	ERTCO	MERCURY	34.5C/46.5 C	WL	WL	INCUBATOR #9	9/9/2014	3/9/2015	0.00	IN USE
2709	FISHER BRAND	SPIRIT-FILLED	-5C/15C	WL	WL	REF./FREEZER #5	9/9/2014	3/9/2015	0.20	IN USE
2880	VWR SCIENTIFIC	MERCURY	35C/46C	WL	WL	INCUBATOR #8	9/9/2014	3/9/2015	-0.10	IN USE
2954	A&M THER. CORP.	SPIRIT-FILLED	-5C/15C	WL	WL	REFRIGERATOR #7	9/9/2014	3/9/2015	0.50	IN USE
2963	A&M THER. CORP.	SPIRIT-FILLED	-5C/15C	WL	WL	REFRIGERATOR #6	9/9/2014	3/9/2015	0.50	IN USE
2995	A&M THER. CORP.	SPIRIT-FILLED	-5C/15C	WL	WL	REFRIGERATOR #10	9/9/2014	3/9/2015	0.40	IN USE
3537	ERTCO	SPIRIT-FILLED	-5C/15C	WL	WL	REFRIGERATOR #8	9/9/2014	3/9/2015	0.30	IN USE
3941	A&M THER. CORP.	SPIRIT-FILLED	-5C/15C	WL	WL	REFRIGERATOR #4	9/9/2014	3/9/2015	0.10	IN USE
3971	ERTCO	COOLER THER	-10C/20C	WL	EH	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.20	IN USE
4282	ERTCO	COOLER THER	-10C/20C	WL	EH	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.20	IN USE
4428	ERTCO	COOLER THER	-10C/20C	WL	OCPW	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.50	IN USE
4495	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #1	9/9/2014	3/9/2015	0.20	IN USE
4637	ERTCO	SPIRIT-FILLED	-10C/20C	WL	WL	INCUBATOR #9	9/9/2014	3/9/2015	0.50	IN USE
5229	ERTCO	COOLER THER	-10C/20C	WL	EH	FIELD/COOLER THERM.	9/9/2014	3/9/2015	-0.20	IN USE
5511	ERTCO	COOLER THER	-10C/20C	WL	EH	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.20	IN USE
5526	ERTCO	COOLER THER	-10C/20C	WL	OCPW	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.00	IN USE
6196	ERTCO	COOLER THER	-10C/20C	WL	WL	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.20	IN USE
6533	ERTCO	COOLER THER	-10C/20C	WL	WL	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.20	IN USE
6670	ERTCO	COOLER THER	-10C/20C	WL	OCPW	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.50	IN USE
6761	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #2	9/9/2014	3/9/2015	0.30	IN USE
6863	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #2	9/9/2014	3/9/2015	0.30	IN USE
6957	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #2	9/9/2014	3/9/2015	0.30	IN USE
7040	ERTCO	MERCURY	20C/50C	WL	WL	INCUBATOR #2	9/9/2014	3/9/2015	0.30	IN USE
7537	ERTCO	MERCURY	35C/46C	WL	WL	INCUBATOR #9	9/9/2014	3/9/2015	0.00	IN USE
8201	ERTCO	COOLER THER	-10C/20C	WL	EH	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.50	IN USE
8420	ERTCO	COOLER THER	-10C/20C	WL	OCPW	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.00	IN USE

8431	ERTCO	COOLER THER	-10C/20C	WL	EH	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.40	IN USE
8436	ERTCO	COOLER THER	-10C/20C	WL	OCPW	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.40	IN USE
8454	ERTCO	COOLER THER	-10C/20C	WL	OCPW	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.00	IN USE
8475	ERTCO	COOLER THER	-10C/20C	WL	OCWD	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.50	IN USE
8497	ERTCO	COOLER THER	-10C/20C	WL	EH	FIELD/COOLER THERM.	9/9/2014	3/9/2015	0.40	IN USE
18428	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #1	9/9/2014	3/9/2015	0.20	IN USE
19426	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #1	9/9/2014	3/9/2015	0.20	IN USE
19845	ERTCO	SPIRIT-FILLED	20C/50C	WL	WL	INCUBATOR #2	9/9/2014	3/9/2015	0.30	IN USE
20093	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #1	9/9/2014	3/9/2015	0.20	IN USE
20166	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #8	9/9/2014	3/9/2015	0.40	IN USE
20368	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #8	9/9/2014	3/9/2015	0.40	IN USE
20424	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #1	9/9/2014	3/9/2015	0.20	IN USE
20461	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #1	9/9/2014	3/9/2015	0.20	IN USE
20502	ERTCO	SPIRIT-FILLED	+18C/50C	WL	WL	INCUBATOR #2	9/9/2014	3/9/2015	0.30	IN USE
20540	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #1	9/9/2014	3/9/2015	0.20	IN USE
112426	ERTCO	SPIRIT-FILLED	+20C/50C	WL	WL	INCUBATOR #1	9/9/2014	3/9/2015	0.20	IN USE
171908	ERTCO	SPIRIT-FILLED	-10C/50C	WL	WL	MAX Q4000	9/9/2014	3/9/2015	0.40	IN USE
172035	ERTCO	SPIRIT-FILLED	-10C/20C	WL	WL	INCUBATOR #10	9/9/2014	3/9/2015	0.50	IN USE
B02-100	ERTCO	MERCURY	28C/44.8C	WL	WL	INCUBATOR #6	9/9/2014	3/9/2015	0.10	IN USE
B02-102	ERTCO	MERCURY	28C/44.8C	WL	WL	INCUBATOR #7	9/9/2014	3/9/2015	-0.10	IN USE
B02-104	ERTCO	MERCURY	28C/44.8C	WL	WL	INCUBATOR #6	9/9/2014	3/9/2015	0.00	IN USE
H01-331	ERTCO	MERCURY	28C/44.8C	WL	WL	INCUBATOR #5	9/9/2014	3/9/2015	-0.10	IN USE
H01-335	ERTCO	MERCURY	28C/44.8C	WL	WL	INCUBATOR #5	9/9/2014	3/9/2015	0.00	IN USE
J01-515	ERTCO	MERCURY	28C/44.8C	WL	WL	INCUBATOR #7	9/9/2014	3/9/2015	0.00	IN USE

6.9. Example of Thermometer Certificate of Calibration (in ELOP Plan)

Service/Calibration Record **Superior Scientific**

Customer No. 20878-WT	Superior Scientific, Incorporated
Contract Date 03/01/13	301 South Raymond Avenue
PM Start Date 03/01/13	Alhambra, California 91801
	24-Hour Tel: 626 281-5588
Equipment Code ERTCO301SR	
Equipment Class 4	
PM Frequency Semi-Annual	
Customer COUNTY OF ORANGE/PUBLIC HEALTH	Department: THERMOMETER
Address 600 SHELLMAKER ROAD	Location INCUB. #1
City NEWPORT BEACH	Contact JOE GUZMAN
State CA	Phone 949/219-0423
Manufacturer ERTCO	Description THERMOMETER (RED FLUID)
Model I-030-1SR	Serial No. 4495
Prospective Maintenance Procedures 01 VISUAL INSPECTION 02 CHECK FLUID COLUMN FOR SEPARATIONS 03 CALIBRATE UNIT THERMOMETER: UNIT THERMOMETER NIST THERMOMETER CERTIFIED THERMOMETER SERIAL No.	
Date of PM 3/29/13 10/2/13 3/19/14 9/9/14 LH LH LH LH 35.0°C 35.0°C 35.0°C 35.0°C 35.0°C 35.0°C 35.2°C 35.0°C 6080672 6080672 6080672 6080672	

6.10. Monthly ELAP Autoclave Validation Checklist (Blank)

ELAP Autoclave Validation Checklist (Monthly)

Month: _____

Media Requisitions

Make sure bottom portion of all requisitions are completely filled out with Autoclave #, Run #, Time in, Time out, Elapsed time, Date made, Made by, and Final pH. If elapsed time is greater than 45 minutes then notify the Micro.

Date																		
Checked																		
Initial																		

Comments _____

Autoclave Operating Temperature (Weekly)

Make sure the Media Room is documenting this. Notify Micro

Date					
Checked					
Initial					

Comments _____

BIOSIGN Spore Test (Monthly)

Make sure that the Media Room is documenting this in the Biological Monitoring Record. Make sure all autoclaves are being checked, the sterilization cycle is 15 minutes, and the results were negative. Notify the Micro of any discrepancies.

Date	
Checked	
Initial	

Comments _____

Sterilization Cycle run time (Performed quarterly by Preventative Maintenance)

Check with the Media Room to see when the next PM is due. Follow through with the Media Room to make sure it gets done. When the PM is performed make sure the sterilization cycle is monitored against a second timing device. If the PM is not due this month, then indicate this on the check list with "ND"

Date Service Due: _____

Date	
Checked	
Initial	

Comments _____

6.11. Quanti-tray Sealer Performance Check (blank)

Quanti-Tray Sealer Monthly Performance Check

Fill a quanti-tray with 1ml Safranin suspended in a 99ml dilution blank

Check for any leakage in-between wells

If any leakage occurs notify a Micro

If no Leakage is present then place a √ in the "OK" column

Date Performed	Analyst	Quanti-tray Lot #	OK	Comments

7. Glassware

7.1. Glassware pH Checklist/SOP (Blank)

Purpose: To spot-check glassware for presence of alkaline or acid residue.
Frequency: Semi-annually.
Procedure: Add a few drops of 0.04% bromthymol blue (BTB) or other pH indicator and observe the color reaction. To prepare BTB, add 16 mL 0.01N NaOH to 0.1g BTB and dilute to 250mL with reagent water.
Results: BTB should be blue-green (in the neutral range).
Reference: SM9020B.3.a.1

	Date Tested	Results	Micro Initials
Graduated Cylinder			
Flask			
Tube with inserts			

7.2. Inhibitory Residue Testing SOP

- 1) Principle
 - a) Certain wetting agents or detergents used in washing glassware may contain bacteriostatic or inhibiting substances requiring 6 to 12 rinsings to remove all traces and insure freedom from residual bacteriostatic action. If pre-sterilized plastic ware is used, test it for inhibitory residues.
- 2) Frequency
 - a) Perform the test upon initial use of washing compound and when there is a change in the formulation used, or a change in the method of washing reusable glassware.
- 3) Procedure
 - a) Wash and rinse 6 petri dishes according to usual laboratory practice and designate as Group A.
 - b) Wash 6 petri dishes as above, rinse 12 times with successive portions of reagent water, and designate as Group B.
 - c) Rinse 6 petri dishes with detergent wash water (in use concentration), dry without further rinsing, and designate as Group C.
 - d) Sterilize dishes in Groups A, B, and C by the usual procedure.
 - e) To test pre-sterilized plastic ware, set up 6 sterile plastic petri dishes, designate as Group D, and proceed.
 - f) Add not more than 1 ml. of a culture of *E. aerogenes* known to contain 50 to 150 colony-forming units to dishes in Groups A through D, and proceed according to the procedure described for the heterotrophic plate count (Section 9215). Preliminary testing may be necessary to obtain the specified count range. To help assure counts within the specified range, inoculate three plates of each group with 0.1 ml and the other three plates of each group with 1 ml.
- 4) Interpretation
 - a) Difference in averaged counts on plates in Groups A through D should be less than 15% if there are no toxic or inhibitory effects.
 - b) Differences in averaged counts of less than 15% between Groups A and B and greater than 15% between Groups A and C indicates that the cleaning detergent has inhibitory properties that are eliminated during routine washing.
- 5) Reference
 - a) SM9020B.3.a.2

7.3. Inhibitory Residue Report Form (Current) – in ELOP Plan

7.4. Inhibitory Residue Report Form (blank)

Date Set-up: _____

Date Read: _____

Set-up By: _____

Read By: _____

Micro Initial: _____

Group A

Normal Washing Procedure

	CFU	CFU	CFU	Avg.
0.1ml				
1.0ml				
Combined Avg. = A				

Group B

Rinsed with reagent grade water

	CFU	CFU	CFU	Avg.
0.1ml				
1.0ml				
Combined Avg. = B				

Group C

Rinsed with detergent wash water

Mfg: _____

Detergent Lot # _____

	CFU	CFU	CFU	Avg.
0.1ml				
1.0ml				
Combined Avg. = C				

Group D

Plasticware

Mfg: _____

Lot # _____

	CFU	CFU	CFU	Avg.
0.1ml				
1.0ml				
Combined Avg. = D				

Calculate:

$\frac{B - A}{B} \times 100 =$		(% difference between routinely rinsed petri dishes and controls) Inhibitory properties are removed if % difference is less than 15%.
$\frac{B - C}{B} \times 100 =$		(% difference between unrinsed petri dishes and controls) Inhibitory properties are removed if % difference is greater than 15%.
$\frac{B - D}{B} \times 100 =$		(% difference between presterilized disposable petri dishes and controls) Indication of inhibitory residue if % difference is greater than 15%.
$\frac{A - C}{A} \times 100 =$		(% difference between unrinsed petri dishes and routinely rinsed petri dishes) Inhibitory properties are removed if % difference is greater than 15%.

8. Reagent Grade Water

8.1. Quality Control of Reagent Grade Water SOP

- 1) Purpose
 - a) To test the quality of reagent grade water obtained from a water purification system (Milli-Q). The Milli-Q water system is located in the Chemistry Lab, at the Main Laboratory in Santa Ana. Conductivity and pH are tested each day a new batch of media is made. Heterotrophic plate count is performed on a weekly basis. Trace metals are tested annually by the Chemistry Lab. (Standard Methods, 20th ed).
- 2) Milli-Q System Operating Procedure
 - a) Press STANDBY/OPERATE button to OPERATE mode. Wait for the digital display to give a reading of greater than 15 mega ohms. The system will usually read 17.8-18.2 mega ohms within a few seconds. If the system displays a lower reading for more than a few minutes, contact the chemists. The QPAK may need to be changed.
 - b) System is kept in the STANDBY and RECIRCULATION modes when not in use
 - c) Use sterile 150 ml beakers to collect the water. Record your results on the Milli-Q Water QC sheet. Notify Microbiologist if the results are not within acceptable range.
 - d) When collecting water for the first run of the day, purge the system for 30 seconds. Enter the purity reading value into the logbook. The reading should be 18.2 mega ohms.
 - e) Remove the blue plastic cap from clear plastic filling bell. Do not remove clear plastic filling bell. Use the supply arm by positioning it at the container and squeezing the trigger. Water will flow into the container. Lock the trigger if desired.
 - f) After collection, release trigger (either by releasing locking latch or trigger arm if not locked). Water flow will stop.
 - g) Press STANDBY/OPERATE button to return system to STANDBY mode.
 - h) The system requires sanitization on a biweekly basis. Using the Millipore procedure. The chemistry section will perform this operation.
 - i) Consult with the Chemistry section if there are any questions.
- 3) Conductivity Procedure (Thermo Orion 115A plus)
 - a) Calibration
 - i) Initiate by pressing the CAL key twice. The “CALIBRATE” indicator will appear at the top center of the display.
 - ii) Press the CAL key until the correct range for your standard appears on the display. The calibration ranges, 199.9 μS , 1999 μS , 19.99 mS and 199.9 mS, may be scrolled through multiple times by pressing the CAL key. When “1.00 cm^{-1} ” or the last chosen cell constant appears on the display, you are back to calibration by cell constant adjustment method.
 - (1) Note: The conductivity standard value should be 10% to 100% of the displayed range of value. For example, 1412 μS would be in the 1999 μS range.

- iii) Immerse the conductivity cell in the KCL conductivity standard, which at 25°C has a conductivity of 1412 μ S. Slightly agitate the cell to remove any air bubbles.
 - (1) Preparing the potassium chloride conductivity standard
 - (a) Dissolve 745.6 mg anhydrous KCL in conductivity water and dilute to 1000 ml in a class A volumetric flask at 25°C and store in a CO₂-free atmosphere
 - iv) Enter the value of the standard by scrolling each digit with the \wedge or \vee key, then accepting the value by pressing the YES key.
 - (1) After the last digit has been entered and accepted by pressing the YES key, the display will go blank. The meter will perform the calibration and return to the measurement mode.
 - b) Measurement
 - c) Press the MODE key to move between conductivity, salinity and TDS.
 - d) Place conductivity cell in the sample. Slightly agitate the cell to remove any air bubbles.
 - e) Allow the reading to stabilize. The conductivity reading in units of either mS (milliSiemens) or μ S (microSiemens) will be displayed.
 - f) After the signal has reached stability the “READY” indicator at the top right of the display will be displayed.
 - g) Acceptable range: <2 μ mhos/cm at 25°C. (Table 9020:I *Std. Methods*) (1 μ mhos/cm = 1 μ S/cm, therefore acceptable range is <2 μ S)
 - h) Record results on QC report form.
 - i) Note: Change conductivity standard every 4 months. Use a fresh conductivity standard if there is a variation of results or visible cloudiness/debris.
 - i) Reference Thermo Orion 115Aplus operation manual.
 - 4) Total Chlorine
 - a) Total Chlorine is tested monthly by the Chemistry department.
 - 5) PH
 - a) Use the pH meter in the media room.
 - i) The pH meter must be calibrated daily with the 4.0 and 7.0 buffers. (10.0 buffer on Mondays only)
 - ii) To calibrate pH meter:
 - (1) If the pH meter is already calibrated, proceed with step 5.2.
 - (2) Pour buffers into their pre-labeled plastic containers.
 - (3) Remove electrode from the pH 7.0 buffer. Rinse with DI water and wipe dry.
 - (4) Insert electrode into the 4.0 buffer and press the calibrate button so that the 4.0 is flashing. Remove electrode once the “measurement” stops flashing.
 - (5) Repeat steps (5.1.2.3) and (5.1.2.4) for 7.0 and 10.0 buffers.
 - b) Remove electrode from the pH 7.0 buffer. Rinse with DI water and wipe dry.
 - c) Select pH mode and place electrode into sample.
 - d) Press READ to start and wait until "measurement" stops flashing.

- i) Acceptable range: 5.5 – 7.5 (Table 9020:I *Std. Methods*)
- e) Record results on QC report form.
- 6) Heterotrophic Plate Count
 - a) The Heterotrophic Plate Count is a procedure for estimating the number of live heterotrophic bacteria in water. The heterotrophic plate count procedure is performed using the pour plate method and is done on a weekly basis (usually Mondays).
 - b) Take a flask of Plate Count Agar media ahead of time to melt the agar in the autoclave. Place the melted agar into a water-bath with holding temperature between 44°C and 46°C.
 - c) Use a large, clean, level work surface. The work area should be disinfected with amphyl before use.
 - d) Label 6 sterile, disposable petri dishes: 2 plates with “1.0 ml”, 2 plates with “0.1 ml”, 1 plate “Sterility” for sterility check, and 1 plate “Air” for air check.
 - e) Collect the water sample in a sterile container from the Milli-Q water system in the Chemistry Lab.
 - f) The lid for the petri dish labeled “Air” should be removed at the beginning of the procedure and closed as soon as the procedure is finished.
 - g) Thoroughly shake the sample 25 times and withdraw 2 ml of sample from the sterile container using a sterile 5ml pipette.
 - h) Transfer 1 ml to each petri dish labeled “1.0 ml”. When discharging the sample, hold pipette at a 45° angle with the tip touching the bottom of the petri dish. Allow 2-4 seconds for the sample to drain from 1 ml graduation mark to the tip of the pipette. Touch pipette tip once against a dry spot on the petri dish.
 - i) Using a sterile 1ml pipette, follow the above procedure and withdraw 0.2 ml of sample and transfer 0.1 ml into each petri dish labeled “0.1ml”.
 - j) Pipette 10 ml of melted Plate Count Agar media into all of the petri dishes using a sterile 10 ml pipette (Air and Sterility included) and mix carefully by rotating the plates in a figure eight motion.
 - i) Pipetting and pouring the agar into the plates must be completed within 20 minutes.
 - k) Allow the plates to solidify for about 10 minutes. Invert plates and incubate at 35°C ± 0.5°C for 48 hours. Limit stacks of plates to 4.
 - l) Transport plates to Water Lab for a Micro I to count colonies
 - i) Count all the colonies on each plate and record the average of each dilution on the QC sheet. If the count is more than 500 CFU/ml, notify Micro I.
 - ii) Read Sterility and Air results and record on QC report form. If sterility or air plates are contaminated, notify Micro I.
 - iii) Acceptable range: <1000 CFU/ml (Table 9020:I *Std. Methods*)
 - m) Reference: *Std.Methods* 18th Edition Sections 9010B and 9215 A &B

8.2. Quality Control of Milli-Q Water Report Log

Quality Control Report Sheet

Type 1 Water (Milli-Q)

Chemistry Analysis

Analyst		
Date Tested		
Analyte Tested	Acceptable Range	Determined Value
pH	5.5-7.5	
Conductance	<2 µmhos/cm	
 <u>Heterotrophic Plate Count (Pour Plate Method)</u> 		
Plate	Plate Count/Duplicate (<1000 CFU/ml; NG = No growth)	
1.0 ml		
0.1 ml		
Air		
Sterility		
Micro Initial		
Date Read		
Comments:		

Quality Control Report Sheet

Type 1 Water (Milli-Q)

Chemistry Analysis

Analyst	
Date Tested	

Analyte Tested	Acceptable Range	Determined Value
pH	5.5-7.5	
Conductance	<2 µmhos/cm	

Heterotrophic Plate Count (SimPlate Method)

Plate	Plate Count (<1000 MPN/ml; NG = No growth)
10.0 ml	
Sterility	
Micro Initial	
Date Read	

Comments:

8.3. Total Chlorine Analysis of Reagent Water SOP

ANALYSIS: TOTAL CHLORINE

Reference: Method 4500-Cl F, DPD Ferrous Titrimetric Method, Standard Methods, SM 19 & 20

Apparatus: 5 ml Buret
Analytical balance
Erlenmeyer flasks, 250 ml

Reagents: Phosphate buffer solution, Ricca Cat. No. 5805-32
Ferrous ammonium sulfate, 0.00282N, Ricca Cat. No. 3144-32
Potassium Iodide crystals
DPD indicator solution, Ricca Cat. No. 2655-16

PROCEDURE:

1. Place 5 ml each of buffer reagent and DPD indicator solution in erlenmeyer flask and swirl to mix.
2. Add 100 ml sample and swirl to mix.
3. Add about 1 g of KI crystals and mix to dissolve. Let stand for 2 min.
4. Titrate with ferrous ammonium sulfate titrant until pink/red color is discharged. Take buret reading before and after titrant addition.

NOTE:

1. When titrating Milli-Q water, color produced is very faint. Place flask on a white surface while titrating.
2. Very low volume of titrant is needed with Milli-Q water. Typically only 1/4 to 1/2 drop of titrant is needed to discharge color.
3. Report chlorine concentration of Milli-Q water via fax to Water lab staff.

Calculate Total Chlorine:

For a 100-mL sample, 1.00 mL standard FAS titrant = 1.00 mg Cl as Cl⁻/L.

8.4. Annual Microbiological Suitability Test of Reagent Grade Water SOP

- 1) Each year the reagent grade water (also called type I water) is sent to E.S. Babcock & Sons Inc. for annual Bacteriological Testing.
- 2) The following are the criteria for sending the type I water to E.S. Babcock & Sons Inc.
 - a) The day before sample is collected, call E.S. Babcock & Sons Inc. at (951) 653-3351 to setup an arrangement for testing the water sample and request a price code.
 - b) On the day of testing, collect the sample directly from Milli-Q tank located in Chemistry lab. Use one-liter sterile flask in media room to collect the sample.
 - c) Sample needs to be refrigerated from the time of collection until the time of delivery in water lab refrigerator.
 - d) Notify lab assistant in central processing to arrange a pick up schedule with the carrier. Let them know that the sample should be kept in cool temperature during delivery time.
 - e) Label the sample with date of collection, time of collection and location of sample (OCPHL).
 - f) Attach a memo to the sample, requesting the bacteriological testing.
 - g) Write a requisition to purchasing; request a payment for professional testing to E.S. Babcock & Sons Inc. Write the price code in the requisition.

8.5. Report from Annual Microbiological Suitability Test (in ELOP Plan)

8.6. Quality Control Report form for Buffered Water used in MF

Media: Buffered Water for MF QC Organisms: Sterility only Incubation Requirements: 35°C± 0.5°, O ₂ , 24 Hrs Procedure: Add 80mL of the buffered water to an 80ml bottle of Nutrient Broth. Check for growth.							
Date in QC	Date out QC	Lot #	Date Made	Date Exp	Sterility	Micro Initial	Comments

8.7. Hardy Diagnostic Reagent Water Specifications (in ELOP Plan)

8.8. Northeast Lab Continental RO Water System SOP (in ELOP Plan)

9. Media and Reagents

9.1. Media QC Summary

Asparagine Broth			
QC Organisms:		(+) <i>Pseudomonas aeruginosa</i>	ATCC 27853
		(-) <i>Escherichia coli</i>	ATCC 25922
Sterility Requirements:			
Hold 5%, or 1 tube from each basket for 48 Hrs. for sterility check.			
✓ for OK indicates no contamination, or unequal filling.			
Incubation Requirements:			
35°C ± 0.5°, O ₂ , within 48 Hrs			
Interpretation:			
(+) <i>Pseudomonas aeruginosa</i> = Greenish fluorescence			
(-) <i>Escherichia coli</i> = No fluorescence			
Azide Dextrose Broth			
QC Organisms:		(+) <i>Enterococcus faecalis</i>	ATCC 19433
		(-) <i>Escherichia coli</i>	ATCC 25922
Sterility Requirements:			
Uninoculated medium should appear light to medium amber and clear.			
Incubate one tube from each lot as a sterility check			
✓ for OK indicates no contamination, or unequal filling.			
Incubation Requirements:			
Inoculate and Incubate at 35 ± 2°C for 18 – 48 Hrs			
Interpretation:			
(+) <i>Enterococcus faecalis</i> = Good Growth			
(-) <i>Escherichia coli</i> = Inhibited Growth			
Bile Esculin Azide Agar			
QC Organisms:		(+) <i>Enterococcus faecalis</i>	ATCC 29212
		(-) <i>Escherichia coli</i>	ATCC 25922
Sterility Requirements:			
Plates should appear medium to dark amber with bluish cast, slightly opalescent.			
INCUBATE ADDITIONAL TUBE FOR STERILITY CHECK			
✓ for OK indicates no contamination, no cracking, unequal filling or excessive bubbles.			
Incubation Requirements:			
Inoculate and Incubate at 35 ± 2°C for 18 – 24 hours			
Interpretation:			
(+) <i>Enterococcus faecalis</i> = Good growth with positive, blackening of the medium			
(-) <i>Escherichia coli</i> = Inhibited growth with negative, no color change			

Brilliant Green Bile Broth (BGBB)

QC Organisms: (+) *Escherichia coli* ATCC 25922
(-) *Staphylococcus aureus* ATCC 25923

Sterility Requirements:

Hold 5%, or 1 tube from each basket for 48 Hrs. for sterility check.

√ for OK indicates no contamination, or unequal filling.

Incubation Requirements:

Inoculate and incubate at 35°C ± 0.5°, O₂, up to 48 Hrs

Interpretation:

(+) *Escherichia coli* = gas in durham tube

(-) *Staphylococcus aureus* = no gas in durham tube

CHROMagar™ ECC

QC Organisms: (+) *Escherichia coli* ATCC 25922
(+) *Klebsiella pneumoniae* ATCC 13883
(-) *Pseudomonas aeruginosa* ATCC 27853

Sterility Requirements:

Incubate additional plates for sterility check

√ For OK indicates no contamination, no cracking, unequal filling or excessive bubbles.

Incubation Requirements:**INOCULATE PLATES USING MEMBRANE FILTRATION METHOD OR STREAK FOR ISOLATION**

Incubate plates at 44°C for 24 hrs in O₂

Interpretation:

(+) *Escherichia coli* = Growth with blue/teal colonies

(+) *Klebsiella pneumoniae* = Growth with red colonies

(-) *Pseudomonas aeruginosa* = Growth with colorless colonies

Colilert-18 Powder (IDEXX)

QC Organisms: (+, +) *Escherichia coli* ATCC 25922
(+, -) *Klebsiella pneumoniae* ATCC 13883
(-, -) *Pseudomonas aeruginosa* ATCC 27853

Sterility Requirements:

No sterility checks needed

Incubation Requirements:

Inoculate and incubate at 35°C, O₂, for 18-22 Hrs

Interpretation:

(+, +) *Escherichia coli* = yellow and fluorescence

(+,-) *Klebsiella pneumoniae* = yellow and no fluorescence

(-, -) *Pseudomonas aeruginosa* = no color and no fluorescence

Colilert MPN (IDEXX)

QC Organisms: (+, +) *Escherichia coli* ATCC 25922
(+, -) *Klebsiella pneumoniae* ATCC 13883
(-, -) *Pseudomonas aeruginosa* ATCC 27853

Incubation Requirements: 35°C, O₂, 18-22 Hrs. No sterility check necessary.

Interpretation:

(+, +) *Escherichia coli* = yellow and fluorescence
(+, -) *Klebsiella pneumoniae* = yellow and no fluorescence
(-, -) *Pseudomonas aeruginosa* = no color and no fluorescence

Colilert P/A (IDEXX)

QC Organisms: (+, +) *Escherichia coli* ATCC 25922
(+, -) *Klebsiella pneumoniae* ATCC 13883
(-, -) *Pseudomonas aeruginosa* ATCC 27853

Incubation Requirements: 35°C, O₂, 18-22 Hrs. No sterility check necessary.

Interpretation:

(+, +) *Escherichia coli* = Yellow and fluorescence
(+, -) *Klebsiella pneumoniae* = Yellow and no fluorescence
(-, -) *Pseudomonas aeruginosa* = No color and no fluorescence

Collection Bottle

QC Organisms: Sterility only

Incubation Requirements: 35°C, O₂, 48 Hrs. Test 5%, or 5 bottles from each box for sterility.
Add 25ml Nutrient Broth to each bottle.

E. C. Media

QC Organisms: (+) *Escherichia coli* ATCC 25922
(-) *Enterococcus faecalis* ATCC 29212

Incubation Requirements: 44.5±0.2°C water bath, 24±2 hrs. Hold 5%, or 1 tube from each basket for 24 Hrs. for sterility check. Check OK for equal filling.

Interpretation:

(+) *Escherichia coli* = Gas in durham tube
(-) *Enterococcus faecalis* = No gas in durham tube

EC Medium with MUG

QC Organisms:	<i>Escherichia coli</i>	ATCC 25922
	<i>Enterobacter aerogenes</i>	ATCC 13048
	<i>Enterococcus faecalis</i>	ATCC 19433

Sterility Requirements:

Uninoculated media should be light amber and clear

Incubate additional tubes for sterility check

✓ for OK indicates no contamination, or unequal filling.

Incubation Requirements:

Inoculate tubes in duplicate. Incubate the first set at $35 \pm 2^\circ\text{C}$ for 24 hrs, and the second set at $44.5 \pm 0.2^\circ\text{C}$. Read Fluorescence under a long-wave UV light

Interpretation:

Escherichia coli: Fluorescence Production, 35°C = good growth with gas, 44.5°C = good growth with gas

Enterobacter aerogenes: No fluorescence, 35°C = good growth with gas, 44.5°C = inhibited growth no gas

Enterococcus faecalis: No fluorescence, 35°C = inhibited growth no gas, 44.5°C = inhibited growth no gas

Enterococcosel™ Agar

QC Organisms:	(+)	<i>Enterococcus faecalis</i>	ATCC 29212
	(-)	<i>Streptococcus pyogenes</i>	ATCC 19615
	(-)	<i>Escherichia coli</i>	ATCC 25922

Sterility Requirements:

✓ For OK indicates no contamination, no cracking, unequal filling or excessive bubbles.

Incubate additional plates for sterility check

Incubation Requirements:

Inoculate and incubate plates at $35 \pm 2^\circ\text{C}$ in O_2 for 18-24 h

Interpretation:

(+) *Enterococcus faecalis* = Growth, blackening around colonies

(-) *Streptococcus pyogenes* = Inhibition (partial to complete)

(-) *Escherichia coli* = Inhibition (partial to complete)

Enterolert Powder (IDEXX)

QC Organisms:	(+) <i>Enterococcus faecium</i>	ATCC 35667
	(-) <i>Aerococcus viridans</i>	ATCC 700406
	(-) <i>Serratia marcescens</i>	ATCC 8100

Incubation Requirements: $41 \pm 0.5^\circ$, O_2 , 24-28 Hrs. No sterility check necessary.

Interpretation:

(+) *Enterococcus faecium* = Blue fluorescence

(-) *Aerococcus viridans* = No fluorescence

(-) *Serratia marcescens* = No fluorescence

Esculin Iron Agar (EIA)

QC Organisms: (+) *Enterococcus faecalis* ATCC 29212
(-) *Escherichia coli* ATCC 25922

Sterility Requirements:

MEDIUM SHOULD APPEAR AMBER WITH A BLUE CAST, SLIGHTLY OPALESCENT WITHOUT PRECIPITATE

✓ for OK indicates no contamination, no cracking, unequal filling or excessive bubbles.

Incubation Requirements:

Remove filter from mE agar plates after 48 hrs growth at $41 \pm 0.5^{\circ}\text{C}$, and place the filter onto EIA. Incubate for 20 min at $41 \pm 0.5^{\circ}\text{C}$

Interpretation:

(+) *Enterococcus faecalis* = black or red/brown precipitate

(-) *Escherichia coli* = No precipitate formed

Ethyl Violet Azide Broth (EVA)

QC Organisms: (+) *Enterococcus faecalis* ATCC 29212
(-) *Escherichia coli* ATCC 25922

Sterility Requirements:

Solution should be light amber, clear to very slightly opalescent.

Incubate uninoculated tubes for sterility check

✓ for OK indicates no contamination, or unequal filling.

Incubation Requirements:

Inoculate and incubate the tubes at $35 \pm 2^{\circ}\text{C}$ for 18 – 48 hours

Interpretation:

(+) *Enterococcus faecalis* = Good growth

(-) *Escherichia coli* = Inhibited growth

Lauryl Sulfate Broth (Domestic)

QC Organisms: (+) *Escherichia coli* ATCC 25922
(-) *Staphylococcus aureus* ATCC 25923

Incubation Requirements:

$35^{\circ}\text{C} \pm 0.5^{\circ}$, O_2 , up to 48 Hrs. Hold 5%, or 1 tube from each basket for 48 Hrs. for sterility check. Check OK for equal filling.

Interpretation:

(+) *Escherichia coli* = Gas in durham tube

(-) *Staphylococcus aureus* = No gas in durham tube

Lauryl Sulfate Broth (Recreational)

QC Organisms: (+) *Escherichia coli* ATCC 25922
(-) *Staphylococcus aureus* ATCC 25923

Incubation Requirements: $35^{\circ}\text{C} \pm 0.5^{\circ}$, O_2 , up to 48 Hrs. Hold 5%, or 1 tube from each basket for 48 Hrs. for sterility check. Check OK for equal filling.

Interpretation:

(+) *Escherichia coli* = Gas in durham tube

(-) *Staphylococcus aureus* = No gas in durham tube

m-EI Agar

QC Organisms: (+) *Enterococcus faecalis* ATCC 29212
 (–) *Escherichia coli* ATCC 25922

Sterility Requirements:

UNINOCULATED MEDIA SHOULD APPEAR LIGHTLY AMBER

Hold 5%, or 10 plates from each batch for 24 ± 2 hours for sterility check.

√ OK if media has no cracking, drying, bubbles, or uneven filling

Incubation Requirements:

Inoculate using membrane filtration method and incubate at $41^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, O_2 for 24 ± 2 hours

Interpretation:

(+) *E. faecalis* = Growth with blue halo

(–) *E. coli* = No growth

m-Endo Agar LES (Lawrence Experimental Station)

QC Organisms: (+) *Escherichia coli* ATCC 25922
 (–) *Salmonella typhimurium* ATCC 14028
 (–) *Staphylococcus aureus* ATCC 25923

Sterility Requirements:

Uninoculated media should appear rose colored, slightly opalescent, with precipitate.

Hold 5%, or 10 plates from each batch for 24 ± 2 hours for sterility check.

√ OK for equal filling, no cracking, bubbles, or dehydration of the media

Incubation Requirements:

Use membrane filter technique and incubate at $35^{\circ}\text{C} \pm 0.5^{\circ}$, O_2 for 24 ± 2 hours.

Interpretation:

(+) *Escherichia coli* = Red colony with metallic green sheen

(–) *Salmonella typhimurium* = Red colony with no metallic sheen

(–) *Staphylococcus aureus* = No growth

m-FC Agar

QC Organisms: (+) *Escherichia coli* ATCC 25922
 (–) *Enterococcus faecalis* ATCC 29212

Sterility Requirements:

Uninoculated media should appear cranberry red, slightly opalescent

Hold 5%, or 10 plates from each batch for 24 ± 2 hours for sterility check.

√ OK for equal filling and no cracking, drying, or bubbles in the media

Incubation Requirements:

Using the membrane filtration method, inoculate and incubate plates at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$, O_2 for 24 ± 2 hours.

Interpretation:

(+) *Escherichia coli* = Growth with blue colonies

(–) *Enterococcus faecalis* = No growth

Modified m-TEC Agar		
QC Organisms:	(+) <i>Escherichia coli</i>	ATCC 25922
	(-) <i>Enterococcus faecalis</i>	ATCC 29212
Sterility Requirements:		
Uninoculated media should appear cream colored.		
Hold additional plates for sterility check.		
√ OK for equal filling and no cracks, drying, or bubbles in the media		
Incubation Requirements:		
Using the membrane filtration method inoculate and incubate at 44.5°C± 0.2°C, O ₂ , for 24 ± 2 hours.		
Interpretation:		
(+) <i>Escherichia coli</i> = Growth with red colonies		
(-) <i>Enterococcus faecalis</i> = No growth		
Nutrient Agar with MUG		
QC Organisms:	(+) <i>Escherichia coli</i>	ATCC 25922
	(-) <i>Enterobacter aerogenes</i>	ATCC 13048
Sterility Requirements:		
Uninoculated media should appear light amber, clear to slightly opalescent without significant precipitate. Incubate additional Plate for sterility check		
√ for OK indicates no contamination, no cracking, unequal filling or excessive bubbles.		
Incubation Requirements:		
After incubation on mENDO agar LES, transfer the membrane to Nutrient agar with MUG. Incubate 18-24hrs at 35 ± 2°C.		
Expose filter surface to long-wave UV light.		
Interpretation:		
(+) <i>Escherichia coli</i> = Fluorescence		
(-) <i>Enterobacter aerogenes</i> = No fluorescence		
Nutrient Broth		
QC Organisms: Sterility only		
Incubation Requirements: 35°C, O ₂ , 48 Hrs. Test 5%, or 1 bottle from each order for sterility.		
Check OK for equal filling.		
Quanti-tray 2000 trays		
QC Organisms: Sterility only		
Incubation Requirements:		
Incubate at 35°C± 0.5°, O ₂ , for 48 Hrs. Test 5%, or 5 bottles from each box for sterility. Add 25ml Nutrient Broth to each bottle.		
Quanti-tray Dilution Blanks (Dilu-lok)		
QC Organisms: Sterility only		
Incubation Requirements:		
Incubate at 35°C± 0.5°, O ₂ , for 24 Hrs. Test 5%, or 1 bottle from each box for sterility. Add 1ml of each water blank to TSB broth. Check OK for even filling.		

R2A Agar

Sterility Requirements:

UNINOCULATED MEDIA SHOULD APPEAR LIGHT AMBER IN COLOR, SLIGHTLY OPALESCENT, WITH A SLIGHT PRECIPITATE

Sterility check only

√ for OK indicates no contamination, no cracking, unequal filling or excessive bubbles.

Incubation Requirements:

Inoculate with tap water samples using the membrane filter method. Incubate at $35 \pm 2^\circ\text{C}$ for 40-72hrs.

Standard Plate Count Agar

QC Organisms:

(+) *Lactobacillus acidophilus* ATCC 11506

(+) *Staphylococcus aureus* ATCC 25923

Sterility Requirements:

Uninoculated media should appear light amber, slightly opalescent, with no precipitate.

Incubate additional plates for sterility check

√ for OK indicates no contamination, no cracking, unequal filling or excessive bubbles.

Incubation Requirements:

INOCULATE AND INCUBATE AT $35 \pm 2^\circ\text{C}$ FOR 18 – 48 HRS

Interpretation:

(+) *Lactobacillus acidophilus* = Good growth

(+) *Staphylococcus aureus* = Good growth

Water Collection Bottle

QC Organisms: Sterility only

Incubation Requirements:

Incubate at $35^\circ\text{C} \pm 0.5^\circ$, O_2 , for 48 Hrs. Test 5%, or 5 bottles from each box for sterility. Add 25ml Nutrient Broth to each bottle.

9.2. Media Requisitions: (in ELOP Plan)

9.2.1. Brilliant Green Lactose Bile Broth, Buffered Water for Membrane Filtration, EC Medium, Lauryl Tryptose Broth, m-ENDO LES Agar, mEI Agar, mFC Agar, Nutrient Broth, Standard Plate Count Agar

9.3. Media QC Sheets:

Media:										
QC Organisms:										
STERILITY REQUIREMENTS:										
Uninoculated media should appear lightly amber										
Hold 5%, or 10 plates from each batch for 24 ± 2 hours for sterility check.										
Check media for cracking, drying, bubbles, or uneven filling										
INCUBATION REQUIREMENTS:										
Inoculate using membrane filtration method and incubate at $41^\circ\text{C} \pm 0.5^\circ\text{C}$, O_2 , for 24 ± 2 hours										
Interpretation:										
(+) <i>E. faecalis</i> = Growth with blue halo										
(–) <i>E. coli</i> = No growth										
Date in QC	Date out QC	(+) Rxn	(–) Rxn	pH	Lot #	Exp. Date	Date made	Ster.	Micro initial	Comments

9.3.1. MF Monthly Media Verification Log, Sample Media QC Sheet Membrane Filtration Media Verification

m-Endo Agar LES Manuf/Lot#					Date Tested	
Colony	Colony Morphology	LTB “+” = gas w/i 48 hrs “-” = no gas w/i 48 hrs	BG “+” = gas w/i 48 hrs “-” = no gas w/i 48 hrs	Result T=typical A=atypical		
TYPICAL – metallic sheen	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
ATYPICAL – non-metallic sheen	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					

m-FC Agar Manuf/Lot#					Date Tested	
Colony	Colony Morphology	LTB “+” = gas w/i 48 hrs “-” = no gas w/i 48 hrs	EC “+” = gas w/i 24 hrs “-” = no gas w/i 24 hrs	Result T=typical A=atypical		
TYPICAL – blue colonies	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
ATYPICAL – gray/green/cream col.	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					

m-EI Agar LES Lot#/Exp					Date Tested	
Colony	Gram Stain (GPC)	Catalase	Bile Esculin	Growth @ 45°C	Growth in 6.5% NaCl	Results T=typical A=atypical
TYPICAL – col w/blue halos	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					

	+	-
Water Bath QC		
Gram Stain QC		
Initial		

9.4. Reagent QC Sheets:

9.4.1. Gram Stain QC, Catalase QC

Reagent Name:	Gram Stain				
QC Organisms:	(+) <i>Staphylococcus aureus</i>		ATCC 25923		
	(-) <i>Escherichia coli</i>		ATCC 25922		
QC +: Gram positive and Gram negative organisms seen on slide.					
Date Tested	Date Made/ Lot #	(+) Reaction	(-) Reaction	Micro Initial	Comments

Reagent Name: Catalase					
QC Organisms:		(+) <i>Staphylococcus aureus</i> ATCC 25923 (–) <i>Group A Streptococcus (S. pyogenes)</i> ATCC 19615			
(+) = Evolution of bubbles/gas (–) = Absence of bubbles					
Date Tested	Date Made/ Lot #	(+) Reaction	(–) Reaction	Micro Initial	Comments

9.5. Media Transport SOP – Procedure for Transporting Media, Cultures and Reagents from Main Laboratory to Water Laboratory in Shellmaker Island

- 1) Media
 - a) Plates
 - i) Pack media in containers that are labeled and sealed (i.e. bins, boxes). Fill with bubble-wrap as needed to prevent plates from moving.
 - ii) Transport media in coolers @ 5°C.
 - iii) Important documentation should accompany such items being transported to Shellmaker
 - b) Tubes
 - i) Pack tubes in metal baskets and fill empty space with paper towels.
 - ii) Place baskets in cardboard boxes and fill with bubble-wrap as needed to prevent tubes from breaking.
 - iii) Seal and label the boxes appropriately.
 - iv) Transport media at room temperature unless it is normally stored at refrigerated temps.
 - v) Transport media requiring refrigeration on ice (5°C).
- 2) Frozen items
 - a) Cryovials
 - i) Pack boxes of frozen cryovials in coolers along with dry ice. Label all boxes appropriately.
 - b) Microtiter plates
 - i) Pack microtiter plates in coolers with dry ice.
 - ii) Make sure all plates are sealed and labeled.
 - c) Water Samples
 - i) Pack bottles in coolers with dry ice (lined with bubble-wrap) to prevent breakage.
- 3) Refrigerated items
 - a) Tubes, Bottles, Reagents
 - i) Pack items in racks or boxes, and place in coolers with ice packs.
 - ii) Make sure all items are tightly sealed and labeled.

- iii) Label infectious material with biohazard stickers.
- 4) Culture plates
 - a) Seal edges of petri dishes with parafilm. Pack dishes into paper bags and transport in a cardboard box labeled with a biohazard sticker.

10. Membrane Filtration

10.1. Membrane Filter Manifold Sterility Check-list

Purpose - To determine manifold sterility at set points throughout the Membrane Filtration process.

Interpretation - Circle the appropriate result as follows:

NG = No growth

ND = Sterility check not performed on media

GR = Any growth at all is to be marked in red and notify a Micro immediately!

Date	Run	m-Endo	m-FC	m-EI	m-TEC	Manifold #	Lot#	Exp.	Micro Initial
	Start	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	Middle (How many? ___)	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	End	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
Date	Run	m-Endo	m-FC	m-EI	m-TEC	Manifold #	Lot#	Exp.	Micro Initial
	Start	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	Middle (How many? ___)	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	End	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
Date	Run	m-Endo	m-FC	m-EI	m-TEC	Manifold #	Lot#	Exp.	Micro Initial
	Start	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	Middle (How many? ___)	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	End	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
Date	Run	m-Endo	m-FC	m-EI	m-TEC	Manifold #	Lot#	Exp.	Micro Initial
	Start	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	Middle (How many? ___)	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	End	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
Date	Run	m-Endo	m-FC	m-EI	m-TEC	Manifold #	Lot#	Exp.	Micro Initial
	Start	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	Middle (How many? ___)	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	End	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
Date	Run	m-Endo	m-FC	m-EI	m-TEC	Manifold #	Lot#	Exp.	Micro Initial
	Start	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	Middle (How many? ___)	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	End	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
Date	Run	m-Endo	m-FC	m-EI	m-TEC	Manifold #	Lot#	Exp.	Micro Initial
	Start	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	Middle (How many? ___)	NG ND GR	NG ND GR	NG ND GR	NG ND GR				
	End	NG ND GR	NG ND GR	NG ND GR	NG ND GR				

10.2. Recreational Water Membrane Filtration SOP

1. Principle

- 1.1. The Membrane Filtration (MF) method provides a direct count of bacterial colonies on the surface of a filter to determine the number of colony forming units (CFU) per 100-ml sample. Total coliforms, fecal coliforms, *Enterococcus* spp., and *Escherichia coli* can be recovered from recreational and domestic waters using the MF procedure.
- 1.2. Total Coliforms typically produce red colonies with a metallic (golden) sheen within 24 hours (at 35°C) on an Endo-type medium containing lactose. This reaction is based on the production of aldehydes from the fermentation of lactose. Non-coliforms produce pink, colorless, blue, white, or red colonies lacking sheen.
- 1.3. Fecal coliforms produce blue colonies of various shades on m-FC medium. Non-fecal coliforms are usually inhibited. However, these organisms can produce gray, green, cream-colored or pale yellow colonies on m-FC medium. Growth and pigment production are based on the selective factors of elevated temperature (44.5°C) and the addition of rosolic acid in the medium, respectively.
- 1.4. *Enterococcus* spp. produce colonies with a blue halo on m-EI medium. Organisms positive for β -D-glucosidase utilize indoxyl- β -D-glucoside in the m-EI medium. The test is also based on hydrolysis of esculin.
- 1.5. *Escherichia coli* (a member of the fecal coliforms group) develop magenta colonies on modified m-TEC medium. The magenta color is due to the enzyme β -D-glucuronidase present in *E. coli* acting on the chromogen (5-bromo-6-chloro-3-indolyl- β -D-glucuronide) found in modified m-TEC medium.

2. Equipment

- 2.1. Filtration units (sterile manifold and funnels)
- 2.2. Sample collection bottles (sterile)
- 2.3. Pipettes: 10-ml and 1-ml (sterile)
- 2.4. Petri dishes 50x12mm
- 2.5. Membrane filter, 47mm diameter, HA 0.45 μ m pore
- 2.6. Forceps with smooth tips (sterile)
- 2.7. Cotton swabs (sterile)
- 2.8. Cotton balls - medium
- 2.9. Incubators (35°C, 41°C, 44.5°C)
- 2.10. Water Bath (44.5°C)
- 2.11. Microscope (binocular dissecting) with 10-15 X magnification and white fluorescent light source
- 2.12. Rinse bottles - squeezable
- 2.13. Colony counter
- 2.14. Vacuum source
- 2.15. Filtering and safety trap flasks (1L & 4L)
- 2.16. Flame source (butane lighters)
- 2.17. Disposable plastic loops (sterile)
- 2.18. Waste discards (biohazard disposal)

- 2.19. Pipette discards
- 2.20. Calculator
- 3. Media and Reagents
 - 3.1. MF Media: m-Endo LES agar, m-FC agar, m-EI agar, and modified m-TEC agar
 - 3.2. Other Media: LTB, BGBB, EC Broth, Bile esculin slants, 6.5% NaCl broth, Brain-Heart Infusion Broth (BHIB)
 - 3.3. 3% Catalase
 - 3.4. 65% Ethanol (EtOH) – cotton balls are saturated with EtOH
 - 3.5. Phosphate buffered water working solution in squeezable rinse bottles.
 - 3.6. Phosphate buffered dilution blanks (90 and 99-ml)
- 4. Quality Control
 - 4.1. Sterility controls for the membrane filters and filtration unit are performed each day of use (see Section 9) and documented in the Log Book.
 - 4.2. Verification is performed on the membrane filtration media (see Sections 14 & 15) and documented in the Log Book.
 - 4.3. Positive and negative control organisms and sterility testing is performed weekly and on each new batch/lot of media. Sterility testing is performed on the rinse buffer. Results are documented in the Media Quality Control Book.
- 5. Logging in Samples
 - 5.1. Use proper safety precautions and personal protective equipment (PPE) when handling laboratory samples.
 - 5.2. Check the sample report form to ensure the appropriate form is being used for the tests requested. Verify that all of the following information has been completed: date, sampler's last name, sample type, location, time collected and test(s) requested. For questions regarding information on the report form, inform a Microbiologist before the sampler leaves the lab.
 - 5.3. All samples tested for compliance need to be received in the lab within six hours of collection time.
 - 5.4. Read and record the cooler temperature on the water lab sign-in sheet (located on the receiving table). The sample collector and the lab assistant logging in the samples should both initial the sign-in sheet.
 - 5.5. The cooler temperature should be < 10°C. Notify a Microbiologist if the temperature is below 2°C or above 10°C.
 - 5.6. Check the last number used from previous run and start with the next number for the current run.
 - 5.7. Number each sample consecutively using pre-printed laboratory labels. Label the report forms with the corresponding lab number.
 - 5.8. On the report form, indicate date and time samples were received, time run, and initial.
 - 5.9. Samples should be tested within 2 hours of receipt in the laboratory. Compliance samples must be tested ≤ 6 hours from time of collection.

Non-compliance samples may be tested within 24 hours of collection time.

6. Selection of Sample Volumes

- 6.1. The sample volume is selected based on the expected bacterial density. The bacterial density may vary based on the type of sample that is received. See Tables 1 & 2 for suggested sample volumes for membrane filtration.
- 6.2. An ideal sample volume will yield 20-80 typical colonies for total coliforms and *E. coli*, 20-60 typical colonies for fecal coliforms and enterococci, but not more than 200 colonies on a membrane filter surface.
- 6.3. Refer to dilution chart posted on the receiving table for standard volumes used for each submitting agency. The dilution chart includes suggested increased dilution volumes for those samples that require endpoints.
- 6.4. Refer to Table 3 for instructions regarding sample volumes and preparing dilutions.

Table 1: Suggested Sample Volumes for Membrane Filter – Total Coliforms

WATER SOURCE	100	50	10	1.0	0.1	0.01	0.001	0.0001
Drinking water	X							
Swimming pools	X							
Wells, springs	X	X	X					
Lakes, reservoirs	X	X	X					
Water supply intake			X	X	X			
Bathing beaches			X	X	X			
River water				X	X	X	X	
Chlorinated sewage				X	X	X		
Raw sewage					X	X	X	X

Table 2: Suggested Sample Volumes for Membrane Filter – Fecal Coliforms/Enterococci/*E. coli*

WATER SOURCE	100	50	10	1.0	0.1	0.01	0.001	0.0001
Lakes, reservoirs	X	X						
Wells, springs	X	X						
Water supply intake		X	X	X				
Natural bathing beaches		X	X	X				
Sewage treatment plant			X	X	X			
Farm ponds, rivers				X	X	X		
Raw municipal sewage				X	X	X		
Feedlot runoff					X	X	X	
Sewage sludge						X	X	X

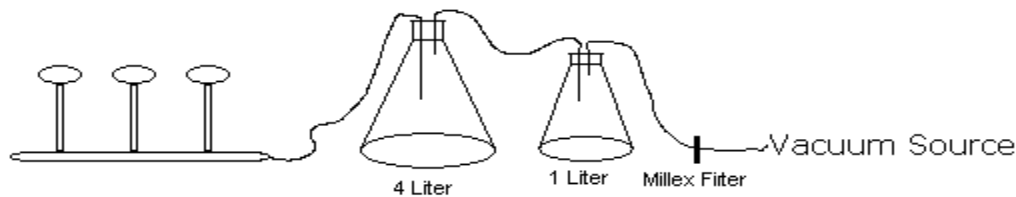
Table 3: Procedures for preparing various sample volumes and dilutions

Sample Volumes	PROCEDURE
10.0 ml	Shake the sample and pipette 10.0 ml of sample directly into funnel.
5.0 ml	Shake the sample and pipette 5.0 ml of sample directly into funnel.
1.0 ml	Pipette 10ml of sample into 90 ml PBS. Shake the 1 st dilution and pipette 10 ml directly into funnel.
0.5 ml*	Pipette 10ml of sample into 90 ml PBS. Shake the 1 st dilution and pipette 5 ml directly into funnel.
0.1 ml	Pipette 10ml of sample into 90 ml PBS. Shake the 1 st dilution and pipette 10 ml into a 2 nd 90 ml PBS. Shake the 2 nd dilution and pipette 10 ml directly into funnel.
0.01 ml	Pipette 10ml of sample into 90 ml PBS. Shake the 1 st dilution and pipette 10 ml into a 2 nd 90 ml PBS. Shake the 2 nd dilution and pipette 10 ml into a 3 rd 90 ml PBS. Shake the 3 rd dilution and pipette 10 ml directly into funnel.
0.001 ml	Pipette 10ml of sample into 90 ml PBS. Shake the 1 st dilution and pipette 10 ml into a 2 nd 90 ml PBS. Shake the 2 nd dilution and pipette 1 ml into 99 ml PBS. Shake the 3 rd dilution and pipette 10 ml into the funnel.
0.0001 ml	Pipette 10ml of sample into 90 ml PBS. Shake the 1 st dilution and pipette 10 ml into a 2 nd 90 ml PBS. Shake the 2 nd dilution and pipette 1 ml into 99 ml PBS. Shake the 3 rd dilution and pipette 1 ml into funnel.

*NOTE: For Environmental Regulatory samples, prepare the 0.5ml dilution by shaking the sample and pipetting directly into funnel.

7. Assembling Filtration Unit
 - 7.1. Ensure that the manifold is connected to a 4 L filtering flask filled with 40-ml of bleach.
 - 7.2. Verify that the 4L filtering flask is connected to a 1 L receiving flask, then to a Millex hydrophobic filter connected to the vacuum source (Figure 1).

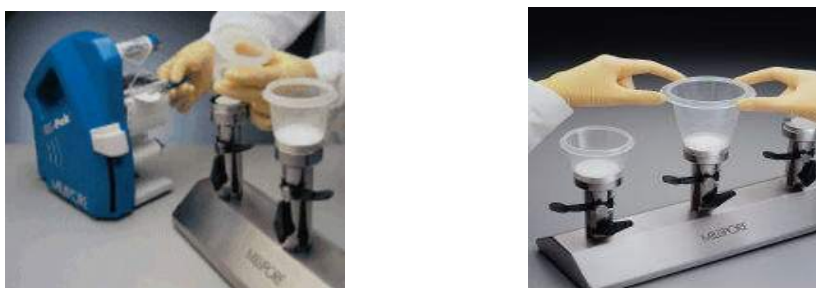
Figure 1



8. Preparing Plates (Labeling)
 - 8.1. Remove plated media from the refrigerator, allowing it to reach room temperature.
 - 8.2. The media are light sensitive. Exposure to light should be kept to a minimum. If media appear discolored, consult with a Microbiologist before labeling the plates.
 - 8.3. Label each plate with the water lab (WL) sample number and dilution tested.
 - 8.4. Plates may be labeled in advance and placed onto a covered rack to minimize exposure to light.
9. Sterility Testing
 - 9.1. For each manifold, label 3 plates each of m-Endo, m-FC and m-EI as follows:
 - 9.1.1. Beginning Sterility –
“Start, Manifold number, Date & Time, Membrane lot number/expiration date”
 - 9.1.2. Middle Sterility –
“Mid, Manifold number, Date & Time, Membrane lot number/expiration date”
 - 9.1.3. End Sterility –
“End, Manifold number, Date & Time, Membrane lot number/expiration date”
 - 9.2. If less than 10 samples are run on a manifold, omit the ‘Middle Sterility’ plates and only set up ‘Beginning’ and ‘End’ sterility plates.
 - 9.3. Be sure to cover plates to prevent exposure to light.
10. Filtration Procedure for Recreational Waters
 - 10.1. Turn on vacuum source. Ensure that the vacuum pump is working properly by testing for suction; place your hand directly over the Microfil support while the valve is in the open position (vertical). The vacuum pump pressure gauge setting should be at 5 in. Hg.
 - 10.2. Open a box of pre-sterilized disposable forceps for use during the procedure.

- 10.3. Turn all the Microfil support valves clockwise (horizontal) to the closed position. Using sterile forceps, wipe the surfaces of the Microfil supports with a cotton ball soaked in 95% EtOH. Dispose the cotton ball and forceps into a red biohazard bag.
- 10.4. Flame the surfaces of the Microfil supports for 3-5 seconds using disposable butane lighter. Allow them to cool by placing valve in the open position before proceeding to the next step. Close valve.
- 10.5. Using a second sterile forceps, place a membrane filter (grid side up) evenly centered over the surface of each Microfil support. Carefully place funnel over each support by snapping the funnels firmly into position (Figure 2).

Figure 2



- 10.6. Sterility testing is performed at the beginning and end of a run and after every 10 samples run on a manifold.
- 10.7. Place sterility plates (pre-labeled) in front of the manifold in the following order: the m-Endo plate should be placed in front of the left Microfil support, followed by the m-FC and the m-EI plates in front of the center and right Microfil supports, respectively. For the 'Beginning' and 'End' sterility, add 20-ml of buffered water (squeeze bottle) to each funnel. Add 100-ml of buffered water for the 'Mid Sterility' testing.
- 10.8. Turn the Microfil support valves to the open position and filter buffered water until all the liquid has passed through the membrane filters.
- 10.9. Starting with the left, close the Microfil support valve. Raise the left funnel to lift. Press the lever on the Microfil support to lift the membrane off the surface (Figure 3). Remove the membrane filter aseptically using sterile forceps. Replace the funnel back onto the Microfil support.

Figure 3



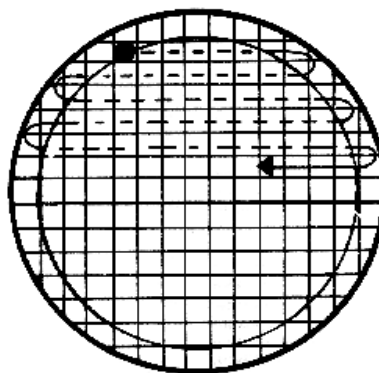
- 10.10. Place the filter from the left Microfil support onto the m-Endo plate, grid side up, with a rolling motion to avoid entrapment of air. Repeat steps 10.8-10.9 for the center and right Microfil supports placing the membrane filter onto the corresponding plates.
 - 10.11. Once the membrane filter has been placed on the media, the plate must be incubated within 20 minutes. Invert the plates (media on top) and incubate as follows:
 - m-Endo – $35.0 \pm 0.5^{\circ}\text{C}$ for 22 to 24 hours
 - m-FC – $44.5 \pm 0.2^{\circ}\text{C}$ for 24 ± 2 hours
 - m-EI – $41.0 \pm 0.5^{\circ}\text{C}$ for 24 hours
 - m-TEC – $35.0 \pm 0.5^{\circ}\text{C}$ for 2 hours, followed by $44.5 \pm 0.2^{\circ}\text{C}$ for 22 ± 2 hours (total incubation time 24 ± 2 hours)
 - 10.12. Repeat steps 10.3 – 10.5 before each new water sample is filtered.
 - 10.13. Start filtration of water samples by placing the pre-labeled media for each sample volume in front of the manifold. For dilutions with a total volume of 5-ml or less, add approximately 10-ml sterile buffered water (squeeze bottle) to each funnel prior to adding sample.
 - 10.14. Mix the water sample by vigorously shaking 25 times in a one-foot arc for 7 seconds.
 - 10.15. Using a sterile pipette, aspirate the highest dilution first and dispense into each of the 3 funnels. For dilutions with a total volume of 5-ml or less, add an additional 10-ml sterile buffered water (squeeze bottle) to ensure adequate distribution of the sample.
 - 10.16. Repeat steps 10.7 – 10.11 (discard funnels and forceps in biohazard bag).
 - 10.17. Each analyst must perform a duplicate analysis on 1 sample or 10% of the daily samples; whichever is greater (see Analyst Precision Criteria SOP).
 - 10.18. Each analyst must document the information for the samples they processed daily in the Membrane Filtration Workload Log (located in the Log Book).
11. Filtration Procedure for Domestic Waters
 - 11.1. Filter 100-ml sample as per Filtration Procedure for Recreational Water (steps 10.1 – 10.9).
 - 11.2. Place filter onto an m-Endo plate and incubate at $35.0 \pm 0.5^{\circ}\text{C}$ for 22 – 24 hours.
 - 11.3. Each analyst must document sample processing information as per step 10.18.
 12. Counting Colonies for Recreational Water Samples
 - 12.1. Use dissecting microscope to view typical colonies. Refer to Table 4 for colony descriptions on each media.

Table 4

Indicator	Media	Ideal Count Range	Typical Colony Morphology (positive)	Atypical Colony Morphology (negative)
Total Coliforms	m-Endo	20-80 typical colonies	Pink to dark-red in color with golden metallic sheen.	Dark red, mucoid, or nucleated without sheen.
Fecal Coliforms	m-FC	20-60 typical colonies	Any shade of blue.	Gray, green, cream, or white with blue/green centers.
Enterococci	m-EI	20-60 typical colonies	Blue halo around the colony, regardless of colony color.	Colonies without blue halo.
<i>E. coli</i>	m-TEC	20-80 typical colonies	Magenta-colored.	White or cream-colored.

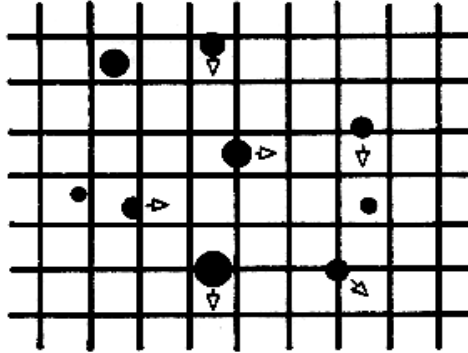
12.2. The grid lines on the membrane act as a guide to counting colonies.
Figure 4 shows how to count plates by scanning from top to bottom, going left to right.

Figure 4



- 12.3. Refer to Figure 5 on how to count colonies that are on the grid lines. (Colonies are counted in the squares indicated by the arrow).

Figure 5



- 12.4. Any plate with < 200 colonies of all types (both typical and atypical colonies) is countable. Record counts of typical colonies on report forms in the appropriate space according to sample volume tested and media used.
- 12.5. For plates that have typical colonies that are countable but > 200 colonies of all types, record counts of typical colonies in the appropriate space with “TNTC” (too numerous to count) in parenthesis next to the count.
- 12.5.1. For example: if there are 50 typical colonies, with > 200 total colonies of all types, record as “50 (TNTC)”.
- 12.6. For plates that have > 200 typical colonies, record as “TNTC” in the appropriate space.
- 12.7. For m-Endo plates that have confluent growth (growth covers the entire filtration area of the membrane and/or colonies are not discrete), then record as “CONF w/sheen” if there is sheen present, or “CONF w/o sheen” if sheen is not present. For m-FC, m-EI and m-TEC plates with confluent growth, record as “CONF” in the appropriate space.
13. Counting Colonies for Domestic Water Samples
- 13.1. Follow counting for recreational water samples (step 12). Count typical colonies on m-Endo media.
- 13.2. Plates that are “TNTC” without sheen or “CONF w/o sheen” must be verified.
14. Membrane Filtration Media Verification for Recreational Samples
- 14.1. MF media verification quality control is performed monthly for m-Endo, m-FC and m-EI to verify identification of typical and atypical colony types from these media.
- 14.2. Document date tested, lot number of media, colony morphology, and verification results in the ‘Membrane Filtration Media Verification’ worksheet (file in the Log Book).
- 14.3. Verification of Total Coliforms from m-Endo

- 14.3.1. Using sterile 10 µl plastic loops pick 10 typical and 10 atypical colonies from m-Endo agar and inoculate directly to Lauryl Tryptose Broth (LTB) and Brilliant Green Bile Broth (BGBB), in this order. Incubate at $35 \pm 0.5^{\circ}\text{C}$ for 24-48 hours.
 - 14.3.2. Positive reactions (gas production) in LTB and BGBB media confirm the presence of Total Coliform bacteria.
 - 14.3.3. If results are atypical, notify Microbiologist to identify the atypical culture and adjust initial counts for that month based on verified data (as per Standard Methods).
 - 14.4. Verification of Fecal Coliforms from m-FC
 - 14.4.1. Pick 10 typical and 10 atypical colonies from m-FC agar and inoculate to EC and LTB media (in this order). Incubate LTB at $35 \pm 0.5^{\circ}\text{C}$ for 24-48 hours and EC at $44.5 \pm 0.5^{\circ}\text{C}$ for 24 hours in a waterbath.
 - 14.4.2. Positive reactions (gas production) in LTB and EC confirm the presence of Fecal Coliform bacteria.
 - 14.4.3. Quality control must be performed for the waterbath each day of use. *E. coli* and *Ent. faecalis* are inoculated into EC media and used as positive and negative controls, respectively, for the waterbath QC.
 - 14.5. Verification of enterococci from m-EI
 - 14.5.1. Pick 10 typical colonies from m-EI media and inoculate into each of the following: Bile Esculin Slant, 6.5% Salt Tolerance Broth (NaCl), and Brain Heart Infusion Broth (BHIB).
 - 14.5.2. Incubate the Bile Esculin slant and the 6.5% NaCl Broth at $35.0 \pm 0.5^{\circ}\text{C}$ for up to 48 hours.
 - 14.5.3. Incubate the BHIB in a waterbath at $44.5 \pm 0.5^{\circ}\text{C}$ for up to 48 hours.
 - 14.5.4. Perform a catalase test by adding catalase reagent to the 48-hour growth on the Bile Esculin Slant. If catalase is positive (bubbles are produced), consult with Supervisor for further work-up.
 - 14.5.5. Perform a gram stain from the growth on the BHIB (see Gram Stain SOP).
 - 14.5.6. Gram stain and catalase quality control must be performed each day of use and recorded in the Quality Control book.
 - 14.5.7. Catalase-negative, gram-positive cocci that grow on bile esculin agar and 6.5% NaCl, and at 45°C in brain heart infusion broth, belong to the *Enterococcus* group.
15. Verification for Domestic Samples
 - 15.1. Non-coliform organisms may produce typical sheen colonies and coliforms may produce atypical colonies without sheen.
 - 15.1.1. With a sterile cotton swab, pick 5 typical and 5 atypical colonies from the membrane filter.
 - 15.1.2. Transfer growth of each colony to EC, LTB, and BGBB broths (in this order).

- 15.1.2.1. Incubate LTB and BGBB broths for 24-48 hours at $35.0 \pm 0.5^{\circ}\text{C}$, and EC broth at $44.5 \pm 0.2^{\circ}\text{C}$ for 24 hours in a waterbath.
- 15.1.2.2. Gas production in LTB and BGBB indicates presence of coliforms. Gas production in LTB, BGBB and EC, indicates presence of fecal coliforms.
- 15.2. The presence of coliforms in cultures showing TNTC w/out sheen, or confluent w/out sheen should be confirmed in the following manner:
 - 15.2.1. Brush entire filter surface with a sterile cotton swab.
 - 15.2.2. Inoculate EC, LTB, and BGBB broths (in this order) using swab.
 - 15.2.3. Incubate LTB and BGBB broths for 24-48 hours at $35.0 \pm 0.5^{\circ}\text{C}$. Incubate EC broth for 24 hours at $44.5 \pm 0.2^{\circ}\text{C}$ (waterbath).
 - 15.2.4. Gas production from LTB and BGBB indicates presence of coliforms. Gas production in LTB, BGBB and EC, indicates presence of fecal coliforms.
16. Calculating and Reporting Recreational Water Results
 - 16.1. If more than one sample volume was tested, use only the count that is in the ideal range to calculate the result.
 - 16.1.1. Compute the result by using the following equation:

$$\text{CFU/100ml} = \frac{\text{No. Typical colonies counted} \times 100}{\text{Volume of sample filtered (ml)}}$$

- 16.1.2. In accordance with Standard Methods and EPA-600/8-78-017.2.8.2, use the count with the highest number of Significant Figures to compute the number of Significant Figures in the answer. If the insignificant digit is less than 5, then replace it with a 0 (3530 = 3500). If the insignificant digit is 5, then round the preceding significant digit to the nearest even number (3450 = 3400; 3550 = 3600). If the insignificant digit is greater than 5, then drop the digit and increase the preceding significant number by 1 (3480 = 3500).
- 16.1.3. Example: The 10-ml plate has a count of 195 and the 0.5-ml plate has a count of 50. Since the 0.5-ml plate has a count that is in the ideal range (20-80 or 20-60) use this count to calculate the result.

$$\text{CFU/100ml} = \frac{50 \times 100}{0.5} = 10,000 \text{ CFU}$$

- 16.1.4. Report as: 10000 CFU per 100ml

- 16.2. If both sample volumes have colony counts that are not in the ideal range and counts are < 200 for all colony types (typical and atypical):
 16.2.1. Compute the result by using the following equation:

$$\text{CFU/100ml} = \frac{\text{Total No. Typical colonies counted} \times 100}{\text{Total Volume of sample filtered (ml)}}$$

- 16.2.2. Example: The 10-ml plate has a count of 195 and the 0.5-ml plate has a count of 10. Both counts are not in the ideal range and have < 200 colonies of all types on each plate.

$$\text{CFU/100ml} = \frac{(195+10) \times 100}{(10 + 0.5)} = 1,952 \text{ CFU}$$

- 16.2.3. Taking Significant Figures into consideration, report as:
1,950 CFU per 100ml

- 16.3. For samples with no colonies present on all plates:
 16.3.1. Compute the result that would have been reported if there had been one colony on the plates, using the equation in 16.2.1
 16.3.2. Example: The 10-ml plate has a count of 0 and the 0.5-ml plate has a count of 0. Calculate the result as if there was 1 colony on the 10-ml plate and place a “less than” sign (<) in front of the result.

$$\text{CFU/100ml} = \frac{1 \times 100}{(10+ 0.5)} = 9.5 \text{ CFU}$$

- 16.3.3. Taking Significant Figures into consideration, report as:
< 10 CFU per 100ml

- 16.4. For samples that have no colonies on one plate while the other is less than the ideal count range:
 16.4.1. Count the colonies on the plate with growth. Then divide the total colonies counted by the total volume filtered.
 16.4.2. Example: The 10-ml plate has a count of 10 while the 0.5-ml plate has a count of 0.

$$\text{CFU/100ml} = \frac{10 \times 100}{10.5} = 95 \text{ CFU}$$

- 16.4.3. Taking Significant Figures into consideration, report as:
100 CFU per 100ml

16.5. For plates with countable typical colonies and >200 of all colony types:

16.5.1. Report as “colony count (TNTC)”

16.5.2. Use the equation in 16.1.1 to calculate the result then place a “greater than or equal to” sign (\geq) in front of the result.

16.5.3. Example: The 10-ml plate is CONF and the 0.5-ml plate has a count of 25(TNTC). Use the count for the 0.5 ml plate to calculate the result.

$$\text{CFU/100ml} = \frac{25 \times 100}{0.5} = 5,000 \text{ CFU}$$

16.5.4. Report as: $\geq 5,000$ CFU per 100ml

16.6. For samples with more than 200 typical colonies on all plates:

16.6.1. Report as: TNTC

16.7. For membranes with confluent growth on all plates:

16.7.1. Total Coliforms (m-ENDO):

16.7.1.1. If “CONF w/ sheen”

Report as: Confluent growth with Coliforms

16.7.1.2. If “CONF w/o sheen”

Report as: Confluent growth without Coliforms

16.7.2. Fecal coliforms (m-FC), enterococci (m-El), and *E. coli* (m-TEC):

Report as: Confluent

16.8. For samples that have no results regardless of the reason (i.e. - technical error, sample broke in transit, or no specimen received)

16.8.1. Report as: No results – (reason)

17. Calculating and Reporting Domestic Water Results

17.1. The EPA Total Coliform Rule for domestic samples requires only a record of coliform presence or absence in a 100-ml sample.

17.2. If no coliform colonies are observed, report the coliform count as “<1 coliform/100ml”.

17.3. For verified coliform counts, adjust the initial count based on the positive verification percentage and report as “verified coliform count/100ml”. Use the following equation to determine percentage of verified coliforms.

$$\% \text{ Verified coliforms} = \frac{\text{number of verified colonies} \times 100}{\text{Total number of coliform colonies subjected to verification}}$$

17.4. For plates with confluent growth or TNTC with at least one detectable coliform colony (which is verified), report as “Total Coliform Positive”.

17.5. For plates with confluent growth or TNTC without detectable coliforms, report as “Invalid”.

17.6. For “Invalid” samples, request a new sample from the same location within 24 hours and select a more appropriate volume to be filtered

per membrane, observing the requirement that the standard drinking water portion is 100-ml.

17.6.1. To reduce the interference of overcrowding, filter 25-ml portions through each of four membranes.

17.6.2. Total coliform counts can be observed on all membranes and reported as CFU/100ml

18. References

18.1. *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1995.

18.2. *EPA Microbiological Methods for Monitoring the Environment*, EPA publication, 1978.

18.3. Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*, EPA Publication EPA/821/R-97/004, March 2000.

10.3. Membrane Filtration Media Quality Control SOP

1. Principle

1.1. For membrane filtration, QC procedures are performed weekly, and when a new batch or lot number of m-Endo, m-FC, m-EI or m-TEC media is received. The following protocol establishes which positive and negative control organisms to use, how to dilute out the organism suspensions to get countable plates, and how to record the results.

2. Equipment

2.1. Refer to Membrane Filtration SOP

3. Reagents

3.1. Media: m-Endo, m-FC, m-EI, m-TEC

3.2. 95 % Ethanol

3.3. Phosphate Buffered Saline (PBS)

3.4. 15-ml Centrifuge tubes

3.5. 2ml Sterile PBS (4 tubes)

3.6. 9.9ml Sterile PBS (12 tubes)

4. Quality Control Organisms

4.1. For m-Endo

4.1.1. Positive Control - *Escherichia coli* ATCC 25922

4.1.2. Negative Control - *Sal. typhimurium* ATCC 14028

4.1.3. Negative Control - *Staph. aureus* ATCC 25923

4.2. For m-FC

4.2.1. Positive Control - *Escherichia coli* ATCC 25922

4.2.2. Negative Control - *Entero. faecalis* ATCC 29212

4.3. For m-EI

4.3.1. Positive Control - *Entero. faecalis* ATCC 29212

4.3.2. Negative Control - *Escherichia coli* ATCC 25922

4.4. For m-TEC

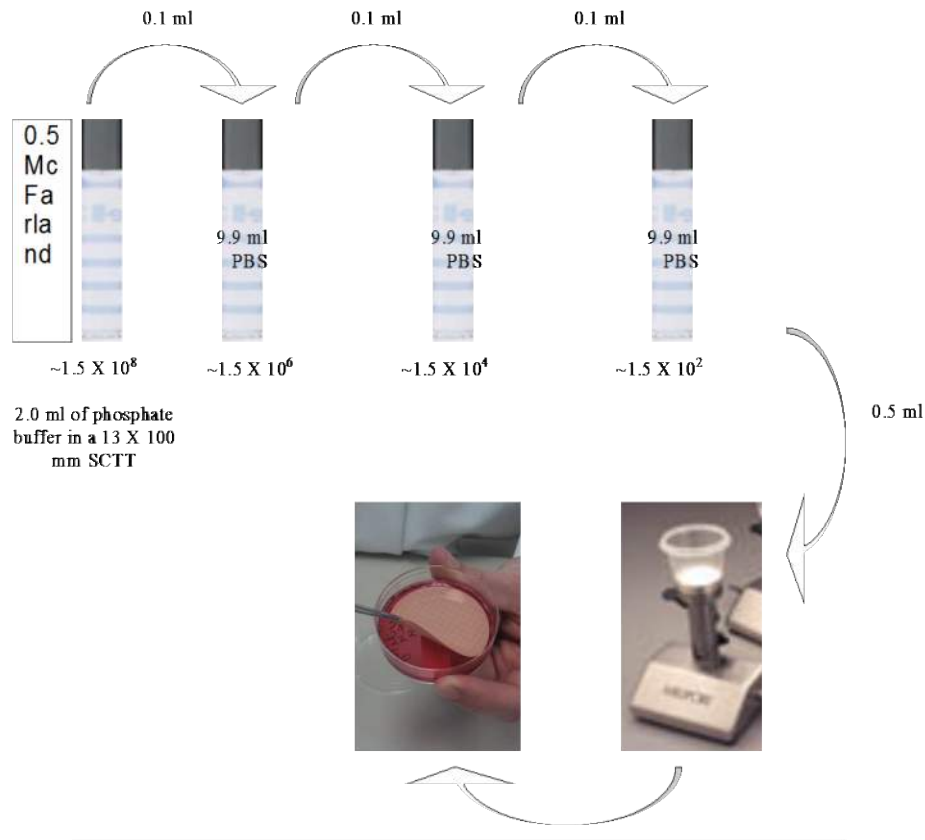
4.4.1. Positive Control- *Escherichia coli* ATCC 25922

4.4.2. Negative Control- *Entero. faecalis* ATCC 29212

5. Procedure
 - 5.1. The membrane filtration media are currently purchased from outside vendors.
 - 5.2. The membrane filtration media, m-Endo, m-FC and m-EI can be made in-house, in the Media Room located at the Orange County Public Health Laboratory.
 - 5.3. Set up the quality control (QC) for each new lot number received.
 - 5.4. The media prepared in-house will be transported to the Water Quality Department in large plastic Tupperware® with bubble-wrap to keep plates from moving. The person receiving the media must also initial the requisition form.
 - 5.5. Select 3 plates of m-Endo media, 2 plates of m-FC media, 2 plates of m-EI media and 2 plates of m-TEC media for the control organisms and 1 plate from each Tupperware® container or 5% of the total shipment to set up sterility controls. Allow the plates to reach room temperature and label them with the control organism and date.
 - 5.6. Label 4 tubes of 2ml Sterile PBS with each of the control organisms (*E. coli*, *S. typhimurium*, *S. aureus*, and *E. faecalis*).
 - 5.7. For each of the control organisms, use three tubes of 9.9-ml of PBS dilution blanks (12 total). Label them with the control organism and the three serial dilutions that will be made (1.5×10^6 , 1.5×10^4 , and 1.5×10^2).
 - 5.8. Use a sterile swab to make a suspension for each of the control organisms to 0.5 McFarland turbidity, using the 0.5 McFarland turbidity standard for comparison. The suspension should have approximately 1.5×10^8 bacteria/ml.
 - 5.8.1. Tightly cap the suspension and vortex the suspension for 10 seconds. Using a 1.0ml pipette, transfer 0.1ml of the 0.5 McFarland suspension to the 9.9-ml of PBS dilution blank labeled as the 1.5×10^6 dilution.
 - 5.8.2. Tightly cap the 1.5×10^6 dilution and vortex the suspension for 10 seconds. Using a 1.0ml pipette, transfer 0.1ml of the 1.5×10^6 dilution to the 9.9-ml of PBS dilution blank labeled as the 1.5×10^4 dilution.
 - 5.8.3. Tightly cap the 1.5×10^4 dilution and vortex the suspension for 10 seconds. Using a 1.0ml pipette, transfer 0.1ml of the 1.5×10^4 dilution to the 9.9-ml of PBS dilution blank labeled as the 1.5×10^2 dilution.
 - 5.9. Tightly cap the 1.5×10^2 dilution and vortex the suspension for 10 seconds. Using a 1.0ml pipette, transfer 0.5ml of the 1.5×10^2 dilution to a funnel containing 10ml buffered water and filter. Refer to membrane filtration procedure.
 - 5.10. Filter the *E. coli* suspension 4 times on 4 separate filters. Transfer the first filter to the m-Endo positive control plate, the second to the m-FC positive control plate, the third to the m-EI negative control plate, and the fourth one to the m-TEC positive control plate (as needed).


- 5.11. Filter the *E. faecalis* suspension 3 times on 3 separate filters. Transfer the first filter to the m-EI positive control plate, the second to the m-FC negative control plate, and the third one to the m-TEC negative control plate.
- 5.12. Filter the *S. typhimurium* and the *S. aureus* suspensions once for each organism. Transfer the filters to the m-Endo negative control plates.
- 5.13. Incubate the plates within 30 minutes as follows:
 - 5.13.1. m-Endo at $35 \pm 0.5^\circ\text{C}$ for 22-24 hours
 - 5.13.2. m-FC at $44.5 \pm 0.2^\circ\text{C}$ for 24 hours
 - 5.13.3. m-EI at $41 \pm 0.5^\circ\text{C}$ for 24 hours
 - 5.13.4. m-TEC at $35 \pm 0.5^\circ\text{C}$ for 2 hours and $44.5 \pm 0.2^\circ\text{C}$ for 22 hours
6. Reporting
 - 6.1. Record results in the QC notebook. Record positive, negative, and sterility results. Indicate the date tested and the date the results were read. Record lot numbers and expiration dates. If QC results are atypical notify the Micro and do not use the media from that lot until discrepancy has been resolved.

10.4. Membrane Filtration Media Quality Control Diagram



10.5. Membrane Filtration Workload Log

Work Log

 County of Orange, Health Care Agency Water Quality Laboratory 600 A Shellmaker Rd Newport Beach, CA 92660 PHONE: (949) 219-0423 FAX: (949) 219-0426		Name: _____ Date: _____ Station: _____						
DUPLICATE		Total Coliforms		Fecal Coliforms		Enterococcus		
		m-Endo Agar LES		m-FC Agar		m-EI Agar		Report Date/Initials
	Vol.	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	
	Laboratory No.	50.0						
		10.0						
		1.0						
		0.5						
		0.1						
	0.01							
	0.001							
DUPLICATE		Total Coliforms		Fecal Coliforms		Enterococcus		
		m-Endo Agar LES		m-FC Agar		m-EI Agar		Report Date/Initials
	Vol.	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	
	Laboratory No.	50.0						
		10.0						
		1.0						
		0.5						
		0.1						
	0.01							
	0.001							
DUPLICATE		Total Coliforms		Fecal Coliforms		Enterococcus		
		m-Endo Agar LES		m-FC Agar		m-EI Agar		Report Date/Initials
	Vol.	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	
	Laboratory No.	50.0						
		10.0						
		1.0						
		0.5						
		0.1						
	0.01							
	0.001							
DUPLICATE		Total Coliforms		Fecal Coliforms		Enterococcus		
		m-Endo Agar LES		m-FC Agar		m-EI Agar		Report Date/Initials
	Vol.	CFU's	CFU/100ml	CFU's	CFU/100ml	CFU's	CFU/100ml	
	Laboratory No.	50.0						
		10.0						
		1.0						
		0.5						
		0.1						
	0.01							
	0.001							
Batch	Sample Arrival Time	Processing Start Time	Processing End Time	Processing Time	Beginning Accession No.	Ending Accession No.	Number of Samples Processed	
1								
2								
3								
4								
5								
			Total Processing Time				Total Sample Processed	

11. Analytical Quality Control Procedures

11.1. Measurement of Method Precision SOP

1. Purpose

- 1.1. Measurement of method precision procedure is used to assess the analysts' precision of testing water following Standard Methods. An analysts' precision criterion will be based on a set of statistical formulas that determine the range of allowable variability in their performance. The precision criterion is determined by performing duplicate analysis on the first 15 samples of a specific test. The value is used as the standard to determine whether the analysts' daily performance is in the allowable range of variability. This procedure needs to be done once a year. Precision testing will be performed daily on 10% of an analysts' workload, or at least one per day.

2. Procedure

- 2.1. Create a folder for precision criterion in the water lab main computer for each analyst. (Share folder\Precision Criteria\Current Analysts). Copy "precision criterion template.xls" and "daily precision template.xls" and paste onto the new analysts' folder.
- 2.2. Perform a duplicate of 15 different samples of each representative sample type (i.e. Domestic, Sewage, and Recreational). Duplicate only the total coliform test on Les-Endo, with each sample type.
- 2.3. Label Les-Endo plates for duplicate analysis before starting the daily run of the samples.
- 2.4. Make a photocopy of the original sample worksheet. Label the photocopy as a duplicate and attach to the original worksheet.
- 2.5. After incubation, read and record the results onto the proper worksheets and use counts that fall between 10 and TNTC. Duplicate copy will be kept as a hard copy in a separate folder for each analyst.
- 2.6. Input the results of the calculated total coliforms, including sample numbers, from the original (D1) as well as the duplicate sample (D2), into the "precision criterion" Excel chart for that analyst (see step 1).
- 2.7. Once the first 15 duplicates of a water type have been completed by the same analyst, the "precision criterion" Excel chart will calculate the Precision Criterion for that analyst. For a detailed explanation of the calculations, please see below.
- 2.8. Enter the value for the Precision Criterion in the "daily precision" Excel chart for each analyst.
- 2.9. Once the Precision Criterion is determined for each analyst, continue with steps 3-5 on a daily basis (daily precision). Perform duplicates on 10% of the samples only, or a minimum of one per day. For example, if 10 samples are to be run, duplicate one total coliform test and if 15 samples are to be run, perform two duplicate total coliform tests.
- 2.10. Input results of daily precision, including sample numbers, in the "daily precision" Excel chart for that analyst, as well as in the *Analytical Quality Control QC Sheet*, located in the Water Lab Log book.

- 2.11. Once the results are entered in the “daily precision” Excel chart the Excel program will calculate the range as either “A” or “U”. “A” represents an acceptable range, whereas “U” represents an unacceptable range. Also, enter the “A” or “U” in the *Analytical Quality Control QC Sheet*. All the results need to be checked and signed by a Micro. If an unacceptable range is determined, notify a Micro as soon as possible. The Micro will take appropriate actions to resolve the situation.
- 2.12. Update each analyst’s Precision Criterion annually by repeating steps 2.1 thru 2.8 using the most recent sets of 15 duplicate results.
3. Calculations
 - 3.1. Calculate precision of duplicate analyses for different type of sample examined (domestic water, sewage water, and recreational waters).
 - 3.2. Prepare duplicate analyses on the first 15 positive samples of a specific type. The same analyst will analyze each set of duplicates. Record duplicate analyses as D₁ and D₂.
 - 3.3. Calculate the logarithm of each result. If either of a set of duplicate results is zero, add 1 to both values before calculating the logarithms.
 - 3.4. Calculate the range (R) for each pair of transformed duplicates as the mean (R) of these ranges.
 - 3.5. Analyze 10% of routine samples in duplicate, transform the duplicates and calculate their range. If the range is greater than 3.27R, analyst variability is excessive. Increased imprecision should be investigated and resolved before making further analyses.
 - 3.6. Sample Calculations:
 - 3.6.1. Sum of R_{log} = 0.0981 + 0.0483 + 0.0627 + + 0.069 + 0.0414 = 0.71889
 - 3.6.2. R = (Sum of R_{log})/ n = 0.71889/15 = 0.0479
 - 3.6.3. (n = the number of samples run)
 - 3.6.4. Precision criterion = 3.27R = 3.27(0.0479) = 0.1566
 - 3.6.5. Therefore anytime the daily precision criteria becomes greater than 0.1566 the data is unacceptable. If it remains below 0.1566, the data remains acceptable.

4. Reference

Standard Methods, 18th Edition, 1992; SM 9020B

11.2. Method Precision Log Sheet

Date of Analysis	Analyst	Laboratory No.	Duplicate Analysis		Acceptance Range	Micro Initial	Corrective Actions if any
			D1	D2			

11.3. Analyst Comparison of Plate Counts SOP

1. Purpose

- 1.1. As per Standard Methods (SM) 20th ed.: “For routine performance evaluation, repeat counts on one or more positive samples at least monthly and compare the counts with those of other analysts testing

the same samples. Replicate counts for the same analyst should agree within 5% and those between analysts should agree within 10%”.

2. Procedure
 - 2.1. Use the m-Endo, m-FC and m-EI plates that are used in the weekly media QC. These plates are prepared by following the MF media QC procedure in the Water Lab SOP.
 - 2.2. All analysts will read the same m-Endo, m-FC and m-EI plates and count the typical colonies for each media. Record this number and all other information on the “Analyst Comparison QC” sheet.
3. Percent Difference Calculation
 - 3.1. The percent difference between analysts is calculated using the “Analyst Comparison QC Sheet” Excel spreadsheet for each type of media. The plate counts for each analyst is entered into the worksheet that automatically calculates the percent difference between each analyst. For example, if there are 4 analysts the difference between analyst A is compared with analysts B, C, and D.
4. Corrective Action
 - 4.1. If the percent difference is greater than 10%, the plate must be re-counted by all analysts as well as the supervisor or senior microbiologist. The main reason for discrepancies in counting total coliforms is due to the differences between analysts in counting “typical” vs. “atypical” colonies. Before recounting the plates, the analysts should discuss and agree upon the specific criteria for discriminating between these colony types to minimize differences in counting.

11.4. Analyst Comparison of Plate Counts Log Sheet

	Date Set-up	Date Read	Name of Analyst	Media	Date Media Made	# Typical Colonies	% Difference				Micro Initial
							A	B	C	D	
A								#####	#####	#####	
B							#####		#####	#####	
C							#####	#####		#####	
D							#####	#####	#####		

11.5 Precision Criterion Worksheet Template

Sample Type: OCEAN/BAY

Analyst:

Sample No.	Date of Analysis	Laboratory No.	Duplicate Analysis		Logarithms of Counts		Range of Logarithms (Rlog = L1-L2)
			D1	D2	L1	L2	
1					#NUM!	#NUM!	#NUM!
2					#NUM!	#NUM!	#NUM!
3					#NUM!	#NUM!	#NUM!
4					#NUM!	#NUM!	#NUM!
5					#NUM!	#NUM!	#NUM!
6					#NUM!	#NUM!	#NUM!
7					#NUM!	#NUM!	#NUM!
8					#NUM!	#NUM!	#NUM!
9					#NUM!	#NUM!	#NUM!
10					#NUM!	#NUM!	#NUM!
11					#NUM!	#NUM!	#NUM!
12					#NUM!	#NUM!	#NUM!
13					#NUM!	#NUM!	#NUM!
14					#NUM!	#NUM!	#NUM!
15					#NUM!	#NUM!	#NUM!

SUM OF Rlog = #NUM!
 MEAN OF Rlog = #NUM!
 PRECISION CRITERION = #NUM!

11.5. Daily Precision Worksheet Template

Sample Type: <u>Ocean/Bay</u>							
Analyst:		Precision criterion: _____					
Date of Analysis	Laboratory No.	Duplicate Analysis		Logarithms of Counts		Range of Logs (Rlog = L1-L2)	Acceptance of Range
		D1	D2	L1	L2		
				#NUM!	#NUM!	#NUM!	#NUM!
				#NUM!	#NUM!	#NUM!	#NUM!

12. Parallel Testing – (Data in QA Manual)

12.1. Comparison between MTF and MF (m-Endo)

12.2. Comparison between MTF and MF (m-FC)

12.3. Comparison between Quanti-tray™ and MF (mEI)

12.4. Evaluation of Enterolert™ and mEI Agar for the Enumeration of Enterococci in Environmental Waters

APPENDIX B

**ORANGE COUNTY WATER DISTRICT LABORATORY
STANDARD OPERATING PROCEDURES**

Detailed Sample Processing Procedure

DNA extraction: Approximately 1000ml of the sample was filtered through 0.4um Whatman Nuclepore Hydrophilic Track-Etch Membranes (Pittsburg, PA.) with a 47mm diameter filter. The sample was poured into a sterile, glass filter apparatus and vacuum filtered through the Whatman filter until a sufficient volume was reached. Sterile, HPLC grade water was filtered using the same method to represent QA/QC of the process. The filters were removed from the apparatus using sterile forceps and placed into a sterile petri dish and stored in the freezer (-20°C) until DNA extraction. Total DNA was extracted from pellet using the UltraClean Soil DNA isolation Kit (MoBio Laboratories Inc., Solana Beach, CA), as specified by the manufacturer. Resulting crude DNA was stored at -20°C.

Primer design: Orange County Water District (OCWD) used custom software to design a specific 16s rRNA gene PCR primer set that targets and amplifies a fragment of the *Bacteroides thetaiotamicron* (*B.t.*) 16s rRNA gene and *Prevotella rumnicola* (*P.r.*) 16s rRNA gene. These primer set was developed for identifying sources of human and rumen fecal contamination, and has been validated, and a provisional application filed (Application No. 60/365,164) containing the sequences.

Bird Primers: Presence/absence and relative concentrations of bird primers were determined by qPCR amplification using primers GFD_HeloR and Hex GFD HeloF as described by Green et.al. 2012.

Quantitative Polymerase Chain Reaction (qPCR): Approximately 2-4ng of total DNA was amplified. Each 100-ul PCR mixture contained 1x PCR buffer with 1.5mM MgCl₂ (Applied Biosystems, ABI, Foster City, CA), each deoxynucleotide triphosphate at a concentration of 200uM (ABI), each *B.t.* primer at a concentration of 0.2uM, and 2.5 U of AmpliTaq gold (ABI). The forward primer was labeled with 5-hexachlorofluorescein (Hex), and both primers were synthesized by IDT (Coraville, IA). GeneAmp PCR system 9600 (ABI) was used for amplification with the following conditions: 11 min hot start at 95°C, and then 35 cycles consisting of 94°C for 1min., 58°C for 1min., and 72°C for 3min., followed by final 10min extension at 72°C. Amplicons were visualized for quality control purposes on a 4% NuSieve agarose gel (BMA, Rockland, ME) in 1x TBE buffer.

Restriction endonuclease digestion: All fluorescent amplicons were partially digested with *DdeI* (New England Biolabs, Beverly, Mass) at 37°C for 30 min. Each 20-ul digestion reaction contained approximately 10-30 ng of PCR products, 10 U of *DdeI* enzymes.

GeneScan Analysis: Presence/absence and relative concentrations of *B.t* and *P.r.* were determined by identifying lengths of the fluorescent amplicons and fluorescent restricted PCR products (T-RF) as determined by electrophoresis with a 310 Genetic Analyzer (ABI) as follows: 2-ul of the amplicons and restricted PCR products were mixed with 12.5-ul of a mixture of deionized formamide (ABI) and Tamara 500 internal size standard (ABI). The mixture was then denatured at 94°C for 5 min and immediately chilled on ice prior to electrophoresis with the automated DNA sequencer in the GeneScan mode. The lengths of the fluorescently labeled

amplicons and T-RFs were determined by comparison with Tamara internal size standard using the Local Southern Method in GeneScan software, version 2.1 (ABI).

References:

Hyatt C. Green, H., Dick, L.K., Gilpin, B., Samadpour, M., and Fields, K.G., 2012. **Genetic Markers for Rapid PCR-Based Identification of Gull, Canada Goose, Duck, and Chicken Fecal Contamination in Water.** *Applied Environmental Microbiology*. 78(2) 503-510.

APPENDIX C

**CLINICAL LABORATORY OF SAN BERNARDINO, INC.
STANDARD OPERATING PROCEDURES**

Clinical Laboratory of San Bernardino, Inc.**Standard Operating Procedure for Colilert Presence / Absence Test for the Determination of Total Coliform and E. coli Bacteria****1. Scope and Application:**

- 1.1 The purpose of this procedure is to allow the analyst to determine the presence of Total Coliform and E. coli bacteria in drinking water samples.

2. Reporting Limit:

- 2.1 Not Applicable.

3. Applicable Matrix and Matrices:

- 3.1 Potable water, source water.

4. Summary of the Method:

- 4.1 This method is used to detect total coliform and E. coli within a potable or source water sample. This is done through IDEXX's patented Defined Substrate Technology.
- 4.2 After the addition of Colilert-24 hour media or Colilert-18 hour media and proper incubation, the samples are checked for color response relative to a comparator purchased from IDEXX.
- 4.3 After the incubation period, if the sample color is darker (yellowish) than the comparator, it must be considered positive for Total Coliform bacteria. If the sample has a slight change of color, (yellowish color), it must be allowed to incubate up to an additional 4 hours to determine the result. (Maximum incubation of 28 hours for Colilert-24, 22 hours for Colilert-18.)
- 4.4 All samples that are positive for Total Coliform must be checked under the UV light for presence of E. coli. If a fluorescent blue color equal to or greater than the comparator is seen, then E. coli is present in the sample.

5. Definitions:

- 5.1 Coliform Bacteria - Bacteria of the family Enterobacteriaceae, commonly found in the intestinal tracts of warm-blooded animals; a rod-shaped, lactose fermenting bacteria.
- 5.2 E. coli Bacteria – A specific coliform bacteria used as an indicator organism of pathogens in fecal coliform testing.
- 5.3 **Enzyme** – In this test the enzyme releases a chromogen such as fluorogen which the UV light produces a fluorescent light.
- 5.4 **MUG** - 4-methylumbelliferyl beta-D-glucuronide that is metabolized to create fluorescence light to indicate E. coli bacteria present in sample.
- 5.5 **Potable Water** – Water that is safe for human consumption, also known as drinking water.
- 5.6 **Present / Absent (P/A)** – Present indicates a positive result, whereas, absent indicates a negative result. No numeric values are associated with results.

6. Contamination and Interferences:

- 6.1 Cracked bottles can lead to interference with a false positive result.
- 6.2 When high chlorine levels are present the sample will turn dark blue when Colilert media is added. Such samples are non-acceptable.

7. Apparatus and Materials:

- 7.1 IDEXX 120 mL sterile plastic bacterial sample bottles
- 7.2 Incubator ($35 \pm 0.5^{\circ}\text{C}$)
- 7.3 Non-sterile latex or vinyl gloves
- 7.4 Safety goggles
- 7.5 Thermometers
- 7.6 Colilert P/A comparator
- 7.7 UV light 365-366 nm
- 7.8 Coliform MUG-negative bacteria culture: *Klebsiella pneumoniae* and *Enterobacter aerogenes*
- 7.9 Coliform MUG-positive bacteria culture: *E. coli*
- 7.10 Non-coliform bacteria culture: *Pseudomonas aeruginosa*
- 7.11 Circulating Water Baths at $44.5 \pm 1.0^{\circ}\text{C}$.

8. Reagents and Standards

- 8.1 Colilert 24-hour media (WP200)
- 8.2 Colilert 18-hour media (WP200-18)
- 8.3 Sterile de-ionized water

9. Sample Collection, Preservation, Shipment and Storage:

- 9.1 Sterile sample bottles are purchased from IDEXX. They are 120 mL plastic bottles. These bottles are tested for sterility by the lab as they arrive with certificate of analysis. They contain powdered sodium thiosulfate to remove chlorine from the sample and allow bacterial growth.
- 9.2 Samples are received in the Bacteriological Department from both the Login Department and the Drivers employed by the lab. They are checked against the corresponding paperwork to ensure complete and correct identification.
- 9.3 The samples are also checked for intactness and temperature. Samples that arrive in broken and/or leaking bottles are immediately poured into another sterile IDEXX bottle and a note is made on the paperwork. Sample volumes less than 97.5 mL are also to be noted on the paperwork, and they are non-acceptable. Samples that are greater than 102.5 mL and have adequate airspace in the bottle to allow for proper mixing are vigorously shaken 25 times and poured off to the range of 97.5-102.5 mL; this will be noted on the paperwork. If the sample is completely filled and has no airspace to allow for proper mixing, it will be transferred to a sterile 150 mL IDEXX bottle. The sample then will be vigorously shaken 25 times and poured off to the range of 97.5-102.5 ; this will be noted on the paperwork.
- 9.4 Samples are to arrive at $\leq 10^{\circ}\text{C}$ with blue ice. If this condition is not observed, it is also noted on the paperwork.

- 9.5 Drinking water samples have a 30 hour holding time. Surface water samples have an 8-hour holding time. If these times are exceeded, it is to be noted on the paperwork and client must be notified for resample.

10. Quality Control:

Due to the size of our incubator, a top thermometer and bottom thermometer are in place to monitor the exact temperature reading. Apply correction factor as needed to each thermometer reading. Refer to thermometer SOP for further instructions. Each sample is labeled with a sticker that indicates setup time for 24 Hr Colilert and 44.5 °C bath in/out times for 18 Hr Colilert. This label has initials of the analysts, and the date. The analysts filling out the paperwork will circle their initials followed by the analysts setting up samples. There are fields for indicating which analyst read the Presence Absence (PA) and at what time.

Colilert-18 Hr Method QC (per lot of media received):

- 10.1 QC includes 1 blank, 2 MUG-negative bacteria cultures of *Klebsiella pneumoniae* and *Enterobacter aerogenes*, 1 non-coliform bacteria culture of *Pseudomonas aeruginosa*, and 1 MUG-positive bacteria culture of *E. coli*.
- 10.2 Prepare QC by taking 5 empty 120 mL IDEXX bottles and fill with sterile de-ionized water to 100 mL level as indicated on the bottle.
- 10.3 Take sterile cotton swab; dip in appropriate culture and plant into the properly labeled bottle.
- 10.4 Add Colilert-18 hour media, cap, and shake at an angle for 25 times. Warm the samples by placing in a $44.5 \pm 1.0^{\circ}\text{C}$ waterbath for 7-10 minutes. Remove the black racks with the samples from the bath and tap on the bench towel 3 times.
- 10.5 Place all QC samples into incubator # 4 and incubate for 18 hours at $35 \pm 0.5^{\circ}\text{C}$.

NOTE: QC NEEDED PER LOT OF MEDIA RECEIVED ONLY

Colilert-24 Hr Method QC:

- 10.6 QC includes 1 blank, 2 MUG-negative bacteria cultures of *Klebsiella pneumoniae* and *Enterobacter aerogenes*, 1 non-coliform bacteria culture of *Pseudomonas aeruginosa*, and 1 MUG-positive bacteria culture of *E. coli*.
- 10.7 Repeat steps 10.2 to 10.3
- 10.8 Add Colilert-24 hour media, cap, and shake at an angle for 25 times.
- 10.9 Place all QC samples into incubator # 4 and incubate for 24 to 28 hours at $35 \pm 0.5^{\circ}\text{C}$.

NOTE: QC NEEDED PER LOT OF MEDIA RECEIVED ONLY

IDEXX Bottles check:

Each lot of bottles received from IDEXX must be checked for sterility and volume. To ensure bottle sterility, add 30 mL of double strength tryptic soy broth in one of the new bottles and incubate for 24 hours at $35 \pm 0.5^{\circ}\text{C}$. The bottle must be checked at 24 hours for positive growth. To ensure volume accuracy, place an empty open IDEXX bottle onto the balance and tare/zero the weight. Carefully add DI water into the bottle and avoid any drops of DI water outside the bottle itself. Fill the bottle up to the

designated 100 mL mark and read the weight. The water weight should be $100 \pm 2.5\text{g}$, which gives a range of 97.5-102.5g.

NOTE: In addition, each LOT must be checked for auto-fluorescence under the UV light.

11. Calibration and Standardization:

- 11.1 Each lot of media is checked for response and sterility using coliform negative, coliform positive and fecal coliform bacterial cultures. The results must be consistent with the media's specifications for response to the various cultures. Refer to Table 2 for expected results.
- 11.2 The incubation time for the test must be measured and recorded carefully. Shortening or lengthening the test will affect the results.

12. Procedures:

- 12.1 Sample Preparation for Colilert 18-Hr Method:
 - 12.1.1 The samples and paperwork are checked for accuracy and completeness. Any discrepancies are to be noted on the paperwork.
 - 12.1.2 The sample is given its own specific laboratory number. The number of the sample is in sequential order, starting with rack 1, number 1. Each rack has 20 positions, therefore holding 20 samples. Sample numbering starts in the upper left hand corner, and moves horizontally to the right and continues on the next row starting on the first column. Tray 2 starts at sample number 21 and goes to sample number 40. Fill up each tray according to the amount of samples received and document the lab number on the copied chain of custody.
 - 12.1.3 Check to see if client requests a heterotrophic plate count (HPC) analytical test. If HPC is needed in addition to the P/A test, its I.D. number is put on the top of the bottle in a red sharpie. Refer to SM 9215B SOP for procedures.
 - 12.1.4 Once samples are given their specific laboratory number, it is then checked to ensure the sample is within the range of 97.5-102.5 mL. If the sample is below 97.5, it is considered non-acceptable and the client is notified to resample. If the sample is above 102.5, it will be vigorously shaken 25 times and poured off to be within the 97.5-102.5 mL range; this is noted on the paperwork. However, if the sample is completely full and has no airspace, it will be transferred to a sterile 150 mL IDEXX bottle to allow for proper mixing. Once the sample is poured into the sterile 150 mL bottle, vigorously shake the sample 25 times and pour the sample back into its original bottle to the 97.5-102.5 range; this is noted on the paperwork.
 - 12.1.5 **Shake each sample vigorously, at an angle for 25 times to be sure the sample is representative before setting up HPC.**
 - 12.1.6 Un-cap IDEXX bottle, add Colilert-18 hr media, re-cap bottle and **re-shake each sample vigorously, at an angle, 25 times to dissolve the media.** Each cap is marked with a line using assigned colored sharpie to indicate media added.

- 12.1.7 Warm the samples by placing in a $44.5 \pm 1.0^{\circ}\text{C}$ waterbath for 7-10 minutes. Remove the black racks containing samples from the bath and tap them on the bench towel 3 times. Incubate samples for 18 hours at $35 \pm 0.5^{\circ}\text{C}$. If sample results show a slight yellow color (less than comparator), incubate the sample for up to an additional 4 hours (22 hours maximum).
- 12.1.8 In the case of a positive result, state regulations require the laboratory to notify the client of all positive results within 24 hours. It is required that an employee of the client is spoken to "in person," not just a message. Record all client notifications in the notification logbook. If the client cannot be reached within 24 hours, notify the appropriate state or county authority.
- 12.1.9 When a sample is positive for Total Coliform, it must be checked under the UV light for presence of E. coli. If a fluorescent blue color equal to or greater than the comparator is seen, then E. coli is present in the sample.
- 12.1.10 For dilutions, use only sterile non-buffered water.
- 12.2 Sample Preparation for Colilert 24-Hr Method:
 - 12.2.1 Repeat steps 12.1.1 to 12.1.4.
 - 12.2.2 **Shake each sample vigorously, at an angle; 25 times to be sure the sample is representative before setting up HPC.**
 - 12.2.3 Un-cap IDEXX bottle, add Colilert-24 hr media, re-cap bottle and **re-shake each sample vigorously, at an angle; 25 times to be sure the sample is representative.** To separate 18-Hr samples from 24-Hr samples, mark the cap and paperwork with "24-Hr." Samples with 24-Hr. media are marked with a red sharpie, "24-Hr" is circled, and placed on bottom shelf of the incubator.
 - 12.2.4 Incubate samples for 24 hours at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. If any samples show a slight yellow, but less than the comparator, incubate for up to an additional 4 hours (28 hours maximum).
- 12.3 Incubator Sample Capacity is 840 samples. The Incubators have 7 shelves each. Each shelf holds a maximum of 6 metal racks, meaning $6 \times 20 = 120$ samples; $7 \times 120 = 840$ samples.

13. Calculations:

- 13.1 Not Applicable.

14. Pollution Prevention and Waste Management:

- 14.1 All positive samples generated for Colilert-18 and Colilert-24 methods are to be sterilized before disposal. Use autoclave container and pour all positive samples inside. Cap and insert into autoclave A for 60 minutes at $121-124^{\circ}\text{C}$. The 60 minutes include 30 minutes sterilization time and 30 minutes exhaust time. Once elapsed time is completed dispose solution directly into the sink, containers into a red autoclavable bag to be sterilized.
- 14.2 All negative samples generated for Colilert-18 and Colilert-24 methods are directly disposed into the sink, container vessels into the trash can.

15. Corrective Actions for Out of Control Data:

- 15.1 Records are kept for samples that are unsuitable for this test and the client is notified.
- 15.2 If samples submitted are colored such as yellow, brown, blue, black, etc., so that results will not be readable, clients will be notified and/or an alternative method utilized.
- 15.3 If samples do not pass valid sample criteria, the client is called with options. (Replace sample within 24 hours)
- 15.4 If Colilert media does not pass quality control measures, the media will not be used and must be replaced. Correct incubation times and temperatures must be ensured. Incubator temperatures must be maintained at $35 \pm 0.5^{\circ}\text{C}$. If these are not maintained, the samples contained within the incubators must be re-sampled, results being invalid. Incubators must be monitored for temperature twice a day, at least four hours apart. Incubation times must also be maintained to ensure correct growth cycles. Samples read early could show limited growth. These lead to incorrect results.

16. References:

- 16.1 Standard Methods for the Examination of Wastewater, 20th ed., APHA, AWWA, WEF. Method SM 9223.
- 16.2 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA 815-B-97-001, March 1997.
- 16.3 Code of Federal Regulations, Vol. 40. 2001.
- 16.4 California Code of Regulations. Title 22. Division 4. Chapter 19.

17. Tables, Diagrams, Flow Charts, Etc:

17.1 Table 1: Media Reagents

Item	Part #	Source/Vendor	Type	Concentration	Solvent
1	WP200	IDEXX	Powder	1 packet/100 mL sample	Water
2	WP200-18	IDEXX	Powder	1 packet/100 mL sample	Water

17.2 Table 2: QC, Positive, and Negative Culture Expected Results

Name	Culture Type	Expected Result	UV light Results
Blank	N/A	A	A
P. aeruginosa	Non-coliform	A	A
E. aerogenes	MUG-negative	P	A
K. pneumoniae	MUG-negative	P	A
Escherichia coli	MUG-positive	P	P

18. Training and Qualification Verification:

18.1 Analyst training record on file with data.

19. Health and Safety:

19.1 Safety equipment for this procedure includes non-sterile latex or vinyl gloves and safety goggles. In addition, welding gloves are required for autoclave procedures due to the extreme heat.

19.2 MSDS are records kept on file to inform all those affected by chemicals for proper handling procedures and vital chemical information.

Department: Microbiology

Method Approved By:

Loc Tran
Department Supervisor

Date: _____

Bob Glaubig
Laboratory Director

Alex Popa
QA/QC Manager

Date: _____

Date: _____

Clinical Laboratory of San Bernardino, Inc.

**Standard Operating Procedure for Total Suspended Solids (Non-Filterable Residue) By
Drying Oven**

1. SCOPE AND APPLICATION:

- 1.1 This method is applicable to drinking, surface and saline waters, domestic and industrial wastes.
- 1.2 The practical range of this determination is 2.5 – 20,000 mg/L (ppm).
- 1.3 This procedure measures the non-filterable residue in a water sample, which is dried at 103° - 105°C.

2. REPORTING LIMIT:

- 2.1 The reporting limit is 2.5 mg/L.

3. APPLICABLE MATRIX AND MATRICES:

- 3.1 This method is applicable to drinking, ground, and selected wastewaters.

4. SUMMARY OF METHOD:

- 4.1 The samples are mixed and then filtered through a glass fiber filter. The particulate matter is dried on the filter at 103°C - 105°C to constant weight. The resultant residue is weighed and calculated as total suspended solids.

5. DEFINITIONS:

- 5.1 Laboratory Control Sample (LCS) – A sample of reagent water that is spiked with a known amount of the analyte of interest and then carried through the entire analytical procedure. The LCS result shows the accuracy of the analytical method.
- 5.2 Method Blank (MBLK) – A volume of reagent water that is treated as a sample and carried through the entire analytical procedure. Analysis of the blank demonstrates if interferences are present in the analytical system, which may influence results.
- 5.3 Total Suspended Solids (Non-filterable Residue) – Those solids which are retained by a glass fiber filter and dried to a constant weight at 103°C - 105°C.

6. CONTAMINATION AND INTERFERENCES:

- 6.1 Samples high in Dissolved Solids, such as saline waters, brines, and some wastes, may be subject to a positive interference. Care must be taken in the washing of the filter and in proper drying temperature to avoid potential interference.

7. APPARATUS AND MATERIALS:

- 7.1 Glass fiber filter disks (Gelman A/E, 47 mm diameter, or equivalent).
- 7.2 Filtration apparatus for glass filter disks.
- 7.3 Filter funnel.
- 7.4 Suction flask to hold samples.
- 7.5 Drying oven at 103°C - 105°C.
- 7.6 Analytical balance (± 0.0001 g).
- 7.7 Desiccator for cooling residue with desiccant.
- 7.8 Foil moisture tins, VWR brand.

8. REAGENTS AND STANDARDS:

- 8.1 Absolute Standards, 100 mg/ampule TSS standard which is used for LCS. Prepare 100 mg/L standard for LCS as mentioned in section 12.7.

9. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE:

- 9.1 Samples are collected in polyethylene bottles and stored at 0 to 6°C.
- 9.2 Analyze samples as soon as possible; 7 day maximum holding time.

10. QUALITY CONTROL:

- 10.1 LCS, Dup, and MBLK are quality control (QC) samples.
- 10.2 The test requires one sample duplicate per batch of ten samples.
- 10.3 Record all standards used in the standards preparation log book and the run log book with reference to the chemical inventory book.
- 10.4 All standards used in the method must be labeled with the standards preparation log book number.
- 10.5 Record all deviations and non-conformances in the run log book.

- 10.6 Method Blank must be within an allowable range of < 2.0 mg/L.
- 10.7 RPD for duplicate samples must be less than or equal to 10%, because the method states "duplicate determinations should agree within 5% of their average weight". (5% from average weight each makes 10% RPD)
- 10.8 The LCS recovery for a 100 mg/L standard must be between 80 and 120% acceptance limit.
- 10.9 The weight loss of 1st difference subtracted from the 2nd difference must be less than or equal to 0.5 mg.

11. CALIBRATION AND STANDARDIZATION:

- 11.1 Not Applicable.

12. PROCEDURE:

- 12.1 Preparation of the glass fiber filter. Always use tongs or forceps to handle filters. Insert the filter with the wrinkled side up into the filtration apparatus.
- 12.2 Apply the vacuum and wash the filter with 20 mL portions of reagent water. Rinse filter three times for a total rinse of 60 mL. Continue the vacuum to remove all the excess water.
- 12.3 Place filters in disposable moisture tins and dry the glass filter for 1 hour in the oven at $104 \pm 1^{\circ}\text{C}$ in moisture tin.
- 12.4 Cool the tins in desiccators for 1 hour.
- 12.5 Label tins with the appropriate pan number and weight. Record the weight of tin + filter in mg under the "1st Init Wt" column.
- 12.6 Return pans and filter to $103^{\circ} - 105^{\circ}\text{C}$ over a 1 hour period.
- 12.7 Remove from oven; cool in desiccators for at least one hour.
- 12.8 Record the weight tin + filter in mg under the "2nd Init Wt" column.
- 12.9 The difference between the 1st and 2nd initial weights must be no more than 0.5 mg.
- 12.10 Prepare a MBLK sample by using 1000 mL of reagent water.
- 12.11 Place a dried filter in the filter apparatus using tongs.

- 12.12 Apply vacuum to the filter and pour the sample through the filter slowly.
- 12.13 Prepare a laboratory control sample (LCS) by adding 100 mg of neat stock standard and bring to volume with 1 L of reagent water. The concentration of the working standard should be 100 mg/L.
- 12.14 Place dried filter in the filter apparatus holder with tongs.
- 12.15 Apply vacuum to the filter and pour the sample through the filter.
- 12.16 Measure the amount of volume to be used, based on appearance and sample history using a pre-cleaned graduated cylinder. For every ten samples a duplicate sample must be analyzed. Smaller sample volumes are used on high TSS samples. The amount of sample volume used will be predetermined by the analyst to yield a minimum of 2.5 mg. For samples that have a history of being less than 2.5 mg a minimum of 1000 mL of sample volume will be used. All samples which do not yield a minimum of 2.5 mg residue must have been analyzed using 1000 mL samples. Sample results which do not meet these requirements will have the reporting limit adjusted based on the sample volume used and will be qualified as: insufficient sample volume was available for compliance analysis.
- 12.17 Place dried filter in the filter apparatus holder with tongs.
- 12.18 Apply vacuum to the filter and pour the sample through the filter. If samples are difficult to filter a smaller sample amount may be used. Choose a sample volume that will yield less than 200 mg and more than 2.5 mg of residue. The residue will be weighed on an analytical balance.
- 12.19 After each sample is filtered, it is rinsed 3 times with 10 mL of reagent water. Allow complete drainage between washings. Remove all traces of water by continuing to apply vacuum after water has passed through.
- 12.20 Once rinsing is completed, remove filter disk using tongs and place in pre-labeled corresponding moisture tins.
- 12.21 Dry the sample in the $104 \pm 1^{\circ}\text{C}$ drying oven for 1 hour; then place samples in the desiccator to cool off for at least 1 hour, which allows the sample to balance temperature and weight. Weigh the filter and tin and record results under the column marked "1st Final Wt."
- 12.22 Repeat the drying cycle until a constant weight is obtained. Weigh the filter + tin and record results under the column marked "2nd Final Wt."

12.23 To calculate TSS results the 2nd difference is used as mentioned below. The weight loss of 1st difference subtracted from 2nd difference must be less or equal to 0.5 mg.

13. CALCULATIONS:

13.1 TSS Result (mg/L) = (Sample Wt./Sample Vol.) x 1000

13.2 Duplicate RPD % = [(Result 1 – Result 2) / AVERAGE] x 100

13.3 LCS % Recovery = (Observed LCS / Theoretical LCS) x 100

14. POLLUTION PREVENTION AND WASTE MANAGEMENT:

14.1 Follow the Clinical Laboratory of San Bernardino, Inc. Waste Disposal Plan SOP.

15. CORRECTIVE ACTIONS FOR OUT OF CONTROL DATA:

15.1 Must re-run all samples if any of the QC samples fail to meet acceptance criteria.

15.2 See QA/QC Manager for proper non-conformance documentation.

16. REFERENCES:

16.1 Standard Methods for the Examination of Water and Wastewater, 20th Ed., Section 2-57. Method 2540D. "Total Suspended Solids Dried at 103° - 105°C" (1998).

17. TABLES, DIAGRAMS, FLOWCHARTS, ETC:

17.1 Not Applicable.

18. TRAINING AND QUALIFICATION VERIFICATION:

18.1 Signature of analyst and trainer on file with data.

19. HEALTH AND SAFETY:

19.1 Not Applicable.

Department: Microbiology	
Method Approved By:	
<div style="text-align: center;"><div>_____</div><div>Loc Tran</div><div>Department Supervisor</div><div>Date: _____</div></div>	
<div style="text-align: center;"><div>_____</div><div>Bob Glaubig</div><div>Laboratory Director</div><div>Date: _____</div></div>	<div style="text-align: center;"><div>_____</div><div>Alex Popa</div><div>QA/QC Manager</div><div>Date: _____</div></div>

Clinical Laboratory of San Bernardino, Inc.**Standard Operating Procedure for the Determination of Metals by
Inductively Coupled Plasma Spectroscopy****1. SCOPE AND APPLICATION:**

- 1.1 This method provides procedures for the determination of dissolved elements in ground water, surface water, waste water and drinking water supplies. It may also be used for the determination of total recoverable element concentrations in these waters and wastewaters.
- 1.2 Dissolved elements can be determined after suitable filtration and acid preservation. Acid digestion procedures are required prior to the determination of total recoverable elements on non-waste water if turbidity is greater than 1. All waste water samples require acid digestion prior to total recoverable analysis. To reduce potential interference, dissolved solids should be < 0.2% (w/v).
- 1.3 Prepared samples may require dilution prior to analysis to avoid physical interferences.
- 1.4 The method is applicable to the following analytes:

Analyte	Chemical Abstract Services Registry Numbers (CAS-No.)	Detection Limit of Reporting (DLR)
Aluminum (Al)	7429-90-5	50 ppb
Barium (Ba)	7440-39-3	100 ppb
Boron (B)	7440-42-8	100 ppb
Calcium (Ca)	7440-70-2	1 ppm
Cobalt (Co)	7440-48-4	10 ppb
Copper (Cu)	7440-50-8	50 ppb
Iron (Fe)	7439-89-6	100 ppb / 40 ppb
Magnesium (Mg)	7439-95-4	1 ppm
Manganese (Mn)	7439-96-5	20 ppb / 4 ppb
Molybdenum (Mo)	7439-98-7	10 ppb
Beryllium (Be)	7440-41-7	1.0 ppb
Cadmium (Cd)	7440-43-9	1.0 ppb
Chromium (Cr)	7440-47-3	10 ppb
Nickel (Ni)	7440-02-0	10 ppb
Silver (Ag)	7440-22-4	10 ppb
Potassium (K)	7440-09-7	1 ppm
Silica (SiO ₂)	7631-86-9	0.5 ppm
Sodium (Na)	7440-23-5	1 ppm
Zinc (Zn)	7440-66-6	50 ppb

- 1.5 When using this method for determination of boron, silica, and aluminum in aqueous samples, only plastic, Teflon or quartz lab ware should be used from time of sample collection to completion of analysis. For accurate determinations of boron in solid sample extracts at concentration below 100 mg/kg, only quartz beakers should be used

in the digestions with immediate transfer of an exact aliquot to a plastic centrifuge tube following dilution of the extract to volume. For these determinations, borosilicate glass must not be used in order to avoid sample contamination of these analytes from the glass.

- 1.6 This method is applicable for the direct analysis of total recoverable analytes in drinking water samples containing turbidity < 1 NTU. Use an unfiltered acid preserved sample for analysis.

2. REPORTING LIMIT:

- 2.1 The Detection Limit for Reporting (DLR) is determined as the lowest limit that can be reported that can be reached by the instrument which is usually 3 times higher than the MDL. Experimentally the value can be repeated within a 40% RPD on consistent basis. Refer to table in Section 1.4 for reference. We are reporting at the state DLR levels.

3. APPLICABLE MATRIX AND MATRICES:

- 3.1 Please refer to Section 1.1.

4. SUMMARY OF METHOD:

- 4.1 This method describes a technique for simultaneous multi element determination of metals and trace elements in solution. The basis of the method is the measurement of atomic emission by an optical spectrometric technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where desolvation and excitation occur. Characteristic atomic-line emission spectra are produced by a radio frequency inductively coupled plasma (ICP). The Perkin Elmer combines a polychromator with a solid-state detector in an integrated system optimized for ICP. Background must be measured adjacent to the analyte lines on samples during analysis. The position used must either be free of spectral interference or adequately corrected to reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is handled automatically by the instrument.

5. DEFINITIONS:

- 5.1 Batch – Defined as 10 samples unless otherwise specified. A batch will contain a MBLK, DLR, LCS, LCSD, Interference check solution, MS, MSD, a beginning and ending calibration verification.

- 5.2 Calibration Blank – A volume of de-ionized water acidified with the same acid matrix as in the calibration standards. The calibration blank is used to calibrate the ICP instrument.
- 5.3 Calibration Standard (CAL) – A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to that analyte concentration. It is prepared from the PDS solution standard.
- 5.4 Dissolved Analyte – The concentration of analyte that will pass through at 0.45 um membrane filter assembly prior to sample acidification.
- 5.5 Laboratory Control Sample (LCS) – An aliquot of reagent water to which known quantities of the method analytes is spiked in the laboratory. The LCS is analyzed exactly like the samples, and its purpose is to determine whether method performance is within acceptable control limits. An SDS is used to spike this solution.
- 5.6 Linear Dynamic Range (LDR) – The concentration range over which the analytical curve remains linear.
- 5.7 Method Blank (MBLK) – An aliquot of reagent water that is treated exactly like a sample including exposure to all glassware, equipment, reagents, and acids that are used with other samples. The MBLK is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents or equipment.
- 5.8 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 5.9 Matrix Spike (MS) and Matrix Spike Duplicate (MSD) – A sample is spiked with a known concentration of analytes at mid-range of calibration. The SDS is used to spike this sample.
- 5.10 Primary Dilution Standard (PDS) – A standard which is used in the lab to prepare the calibration standards only.
- 5.11 Secondary Dilution Standard (SDS) – A standard which is used to prepare LCS, MS and MSD samples. It is a different lot from PDS and is referred to as a second source standard.
- 5.12 Spectral Interference Check Solution (SIC) – A solution of selected method analytes of high level concentrations which is used to evaluate the procedural routine for correcting known inter-element spectral interferences with respect to a defined set of method criteria.

- 5.13 Stock Standard Solution – A concentrated solution containing one analyte purchased from a reputable commercial source. Stock standard solutions are used to prepare PDS and SDS and other analyte solutions.
- 5.14 Total Recoverable – The concentration of an analyte determined in an unfiltered sample following treatment by refluxing with hot, diluted mineral acid.

6. CONTAMINATION AND INTERFERENCES:

Spectral Interference:

- 6.1 Can be categorized as an overlap of a spectral line from another element: unresolved overlap of molecular band spectra; background contribution from continuous or recombination phenomena; or background contribution from stray light from the line emission of high concentration elements. The first of these effects can be compensated by utilizing a correction of raw data. The second effect may require selection of an alternative wavelength. The third and fourth effects can generally be compensated by a background correction adjacent to the analyte line.
- 6.2 Spectral overlaps may be avoided by using an alternative wavelength, using multiple wavelengths for each element in your method, or can be compensated for by equations that correct for inter-element contributions, which involves measuring the interfering elements. These interferences will produce false-positive or false-negative determinations and be reported as analyte concentrations. Users may apply inter-element correction factors determined on their instruments to compensate off-line or on-line for the effects of interfering elements. When inter-element corrections are applied, there is need to verify their accuracy by analyzing spectral interference checks.

Physical Interferences:

- 6.3 Are generally considered to be effects associated with the sample nebulization and transport processes. Such properties as change in viscosity and surface tension can cause significant inaccuracies especially in samples that may contain high dissolved solids and/or high acid concentrations. If these types of interferences are operative, they must be reduced by sample dilution and/or utilization of standard addition techniques. Another problem which can occur from high dissolved solids is salt build up at the tip of the nebulizer. This affects aerosol flow rate causing instrumental drift. Wetting the argon prior to nebulization, use of a tip washer, or sample dilution has been used to control this problem. Also, it has been reported that better control of the argon flow rate improves instrument performance. This is accomplished with the use of mass flow controllers.

Chemical Interferences:

- 6.4 Are characterized by molecular compound formation, ionization effects and solute vaporization effects. Normally these effects are not pronounced with the ICP technique, however, if observed they can be minimized by careful selection of operating conditions (i.e. incident power, observation position, etc.), by buffering the sample, matrix matching or standard addition procedures. These types of interferences can be high dependent on matrix type and the specific element.
- 6.5 Serial Dilution – If the analyte concentration is sufficiently high (a factor of 10X the MDL or greater after dilution), an analysis of a dilution should agree within 10% or the original determination. If not, a chemical or physical interference should be suspected.
- 6.6 Analyte addition – A post digestion analyte addition added at a minimum level of 20X the MDL (maximum: 100X) to the original determination should be recovered within established control limits. If not, a matrix effect should be suspected.
- 6.7 Wavelength scanning of analyte line region - Can be performed to detect potential spectral interferences.
- 6.8 Selection of Analyte Wavelength – The most important parameter in the development of an analytical method is the analyte wavelength. The instrument covers the range of wavelengths 165-782 nm and has a resolution of 0.005 – 0.006 nm. The items discussed below should be taken into consideration before making the selection.
- 6.8.1 Nature of Transition – The spectral lines observed in ICP spectroscopy originates from neutral and singly ionized forms of the elements. It is preferable to employ ion lines whenever other considerations permit as they are usually less affected by small changes in the operating conditions of the plasma. Some elements however do not exhibit ion lines of useful intensity two of which are aluminum and boron.
- 6.8.2 Background equivalent concentration (BEC) and Detection Limits is employed in emission spectroscopy to compare the signal to background ratio of the various emission wavelengths of an element. It is defined as the intensity of the plasma background at the selected wavelength. The instrument manual describes how to calculate these for each wavelength. Detection limits can be determined from a BEC. A reasonable detection limit can be calculated by dividing the BEC by 10.
- 6.8.3 Linear Working Range – The upper limits of the linear working range for each element wavelength may vary. If the element concentrations exceed the linear working range of a wavelength it may desirable to utilize a less sensitive wavelength or make an appropriate sample dilution.

- 6.8.4 Interference Equivalent Concentration (IEC) – The IEC is defined as the intensity change, expressed as milligrams of analyte per liter of solution resulting from the analysis of solution containing 1000mg/L of interferant of the analyte wavelength. The IEC indicates the degree of background interference to be expected at a particular wavelength for a particular matrix component. The Optima 5300 has a special program for setting up and testing IEC corrections. These values can be found in the wavelength calibration tables.
- 6.8.5 Memory Effects – Memory interferences result when analytes in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the uptake tubing to the nebulizer and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with rinse blank between samples. The possibility of memory interferences should be recognized with an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for particular element must be estimated prior to analysis.

7. APPARATUS AND MATERIALS:

- 7.1 Perkin Elmer Optima 5300 DV simultaneous inductively coupled plasma-atomic emission spectrometer (ICP-AES).
- 7.2 Dell Personal Computer.
- 7.3 Perkin Elmer software, WinLab32.
- 7.4 Perkin Elmer S10 auto-sampler with trays.
- 7.5 Polyscience Recirculator cooling system.
- 7.6 Peristaltic pump tubing, Blk/Blk for sample.
- 7.7 Peristaltic pump tubing, red/red for waste line.
- 7.8 A 50 mL plastic centrifuge tubes.
- 7.9 15 mL plastic centrifuge tubes.
- 7.10 Argon gas supply, liquid gas pack, high purity grade (99.9%). Argon gas supply pressure should be 80 psi. Compressed air at 80-100 psi as a shear gas.
- 7.11 25, 50, 100 mL volumetric flask.
- 7.12 Brother HL-1440 Laser Printer.

7.13 100 – 1000 μ L – Mechanical pipettor with disposable tips.

8. REAGENTS AND STANDARDS:

NOTE: Only high-purity reagents should be used whenever possible. All acids used for this method must be of ultra pure high purity grade.

8.1 Nitric acid, concentrated – Trace Metals.

8.2 HCl acid, concentrated – Trace Metals.

8.3 Reagent water – For all sample preparation and dilutions, de-ionized water required.

8.4 Stock Standard solutions – Purchased from Absolute Standards in concentrations of 1000 μ g/mL containing metal elements.

8.5 Mixed calibration solutions – Prepare mixed CAL solutions by combining appropriate volumes of the stock standard solutions in volumetric flasks. Refer to Table 3 in Section 17.

8.5.1 Add concentrated nitric acid first to a small volume of water in the flask so that the final concentration of nitric acid will be 2%. Then add the aliquots of stock standard and finally dilute to volume with de-ionized water.

8.5.2 Transfer the freshly prepared CAL solutions to clean polyethylene bottles for storage and label with date, concentration and name. Fresh mixed CAL solutions should be prepared as needed with the realization that concentrations can change on aging. The CAL solutions must be initially verified using a quality control sample.

8.6 Blanks – Three types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, a laboratory reagent blank is used to assess possible contamination from the sample preparation procedure and a rinse blank is used to flush the instrument uptake system and nebulizer between standards and samples to reduce memory interferences.

8.6.1 Calibration Blank (CB) – Prepare by diluting a mixture of 20 mL of concentrated Nitric acid to 1000 mL with de-ionized water. Store in a plastic bottle.

8.6.2 Method Blank (MBLK) – Contains all the reagents in the same volumes used in processing the samples. The MBLK must be carried through the entire preparation procedure and analysis scheme. The final solution should contain the same acid concentrations as sample solutions for analysis.

- 8.6.3 Rinse Blank – Prepare this wash solution by rinsing the zero position cup with reagent grade water and filling with reagent grade water and adding 4 mL of nitric acid.
- 8.7 Laboratory Control Sample (LCS) – These solutions are prepared from a source external to the laboratory. Follow the instructions for preparing these solutions provided by the supplier of the samples. Refer to Table 2 in Section 17.
- 8.8 Detection Limit for Reporting (DLR) – Refer to Table 1 in Section 17.
- 8.9 Spectral Interference Check (SIC) solution aka Interference Check Solution – Follow the instructions provided for preparing and analyzing the interference check solution. When inter-element corrections are applied, SIC solutions are needed.
- 8.9.1 Containing concentrations of the interfering elements at levels that will provide an adequate test of the correction factors. SIC solutions containing Fe 10 mg/L, Al 25 mg/L, Ca 100 mg/L, Mg 25 mg/L Ba, Co, B, Zn, Cu, Mn, Mo all 1.0 mg/L should be prepared in the same acid mixture as the standards and stored in Teflon bottles.
- 8.9.2 If the correction routine is operating properly, the determined apparent analyte concentration from analysis of each interference solution should fall within a specific concentration range bracketing the calibration blank. This concentration range is calculated by multiplying the concentration of the interfering element by the value of the correction factor being tested and dividing by 10. If after subtraction of the calibration blank the apparent analyte concentration is outside this range, a change in the correction factor of more than 10% should be suspected.
- 8.9.3 The cause of the change should be determined and corrected and the correlation factor should be updated.
- Note: The SIC solution should be analyzed more than once to confirm a change has occurred with adequate rinse time between solutions and before subsequent analysis of the calibration blank.
- 8.9.4 The correction factors are tested on a daily basis at the start and end of analysis.

9. SAMPLE COLLECTION, PRESERVATION, SHIPMENT, AND STORAGE:

- 9.1 Samples that cannot be acid preserved at the time of collection because of sampling limitations or transport restrictions should be acidified with nitric acid to pH<2 upon receipt in the laboratory. Following acidification, the sample should be held for 24 hours.

- 9.2 For the determination of total recoverable elements in aqueous samples, acidify with nitric acid at the time of collection to pH<2. The sample should not be filtered prior to digestion.
- 9.3 For non-waste water samples, after the 24 hour acidification, the sample turbidity is tested and samples with turbidity >1 NTU are digested. Before analysis, the pH is checked to ensure pH<2. If pH>2, samples go back to 24 hour acidification process.
- 9.4 For wastewater samples, all samples are acid digested for analysis.
- 9.5 Digest MBLK, LCS, MS and MSD along with samples to be digested.

10. QUALITY CONTROL:

- 10.1 Record all standard preparation in record logbooks at all times. Trace-ability is a requirement for quality control, (QC).
- 10.2 Run a MBLK, DLR, LCS, interference checks, beginning and ending instrument performance checks in each batch of 20 samples. (Currently we are running the MBLK and LCS every ten samples – 04152011- BG and AP)
- 10.3 Run an IPCC, MS, and MSD in each batch of 10 samples.
- 10.4 MBLK must be less than 2.2 times the MDL and below the DLR.
- 10.5 Analysis of the calibration blank should always be less than the DLR but greater than the lower 3-sigma control limit of the calibration blank.
- 10.6 An MDL shall be established for each analyte using reagent water (blank) fortified at a concentration of two to five times the estimated detection limit. To determine MDL values, take seven replicate aliquots of the fortified water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (s)$$

Where: (t) = value for 99% confidence level and a standard deviation estimate with n-1 degrees of freedom is, t = 3.14 for seven replicates.
(s) = Standard deviation of the replicate analysis.

- 10.7 The LCS Recovery must fall within 85 – 115% recovery. The LCS is spiked at the same concentration as the MS and MSD using a secondary source standard. The DLR recovery should be 80 to 120%.

- 10.8 The MS and MSD recovery must fall within 70 – 130% recovery. Matrix Spike RPD must be $\leq 30\%$. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C_s - C}{S} \times 100$$

Where: R = percent recovery

C_s = fortified sample concentration

C = sample background concentration

S = concentration equivalent fortifier added to water sample

- 10.9 Initial Demonstration of performance – Run four LCS samples at mid-range using a second source standard. Accuracy and precision is then determined to see if methodology is being followed.
- 10.10 Interference checks must be monitored at the beginning and at the end of each analytical run meeting the acceptance criteria of $\pm 10\%$.

11. CALIBRATION AND STANDARDIZATION:

- 11.1 A 2 point calibration including a calibration blank is run daily. Refer to Table 3 in Section 17 for reference in calibration preparation procedures.
- 11.2 Before calibrating, the instrument must be started and warmed up for about 30 minutes, and then the alignment is done using 1.0 mg/L of Mn standard for axial and 10.0 mg/L for radial.
- 11.3 Instrument's calibration routine will generate the calibration curve. The calibration curve should be checked for linearity before analyzing any samples to ensure the correlation coefficient (r) is within the acceptable range of at least 0.995 or better.
- 11.4 Linearity check is verified using a calibration verification standard, ICV. The calibration verification standard is run directly following the calibration and must be within $\pm 5\%$ of the concentration chosen at all times. Once the calibration is verified, the continuing calibration verifications run every 10 samples (and at the end of the analysis) should all read within $\pm 10\%$ of their actual concentration. The analyst should also use their experience in interpreting the linearity data.

12. PROCEDURE:

- 12.1 Instrument Start-up and Warm-up Procedures: Click on plasma icon and turn on the plasma.

Interlocks: The following interlocks must be satisfied in order to ignite the plasma. If any of these interlocks are interrupted while the plasma is on, the plasma will automatically be shut down. Argon pressures from the torch must be correct. Argon

pressure leaving the tank must be at least 100 psi. Cooling water must be cooling the RF coil.

12.1.1 First check the water, air and argon supply.

12.1.2 Open door to check torch and all tubing, close the door when done.

12.1.3 Click on WinLab32 icon to access win lab software and turn on the ICP instrument.

12.1.4 Click on Wrkspc and select align ICP and align Torch viewing position by selecting Axial and using 1.00 ppm Mn standard and click align view. Repeat it for Radial and use 10.0 ppm Mn standard.

12.1.5 Click on Wrkspc icon which opens workspace file and select Auto ICP.

12.1.6 Click on Method to open Methods file and Select a method to run.

12.1.7 Click Saminfo to open Sample information Editor. Fill up the parameter like A/S location and sample ID and save as Method Type batch number and your initials.

12.1.8 Go to the automated analysis control and open Sample information file you have saved. Open results data set name. Under select results data set change the name of data set as Method Type batch number and your initials and then click OK.

12.1.9 Click on open Methods in list and print Log during analyses.

12.1.10 Under automated analysis control, click on analyze and then rebuild list. Then click on analyze all. It will start the run, i.e. Calibration, QC and samples.

12.1.11 Method of standard additions is used. The standards scandium and yttrium are added in samples using the pump. This technique compensates for expansion or contraction of an analyte signal by a matrix.

12.2 Digestion Procedures:

NOTE: Digestion needed for samples where turbidity is >1 NTU and for all wastewater samples. Proper digestion will leave approximately 10-20 mL of sample remaining after 4.5 hours.

12.2.1 Fill disposable digestion cup at the 100 mL mark with 100 mL of sample.

12.2.2 Properly label each digestion cup with respective sample ID.

12.2.3 Place on clear plastic tray.

12.2.4 Add 1 mL of concentrated HNO₃ to each sample needing digestion. Try not to splatter.

12.2.5 Add 0.5 mL of concentrated HCl to each sample needing digestion. Try not to splatter.

12.2.6 Place plastic tray with all samples on the digestion block.

12.2.7 Set to digest @ 95°C for 4.5 hours; document on the digest log that the digest block temperature remains below 85°C. The beaker should be covered with an elevated watch glass to prevent sample contamination from the fume hood environment. DO NOT BOIL.

12.2.8 Carefully remove plastic tray and allow the samples to cool.

12.2.9 Bring the remaining sample up to 100 mL with de-ionized water and mix well. Allow any undissolved material to settle overnight.

12.3 Sample Preparation Procedures – Dissolved Analysis:

12.3.1 Label each disposable test tube with respective sample ID.

12.3.2 Filter each sample using 0.45 µm filters, and preserve with 0.5 mL nitric acid per 50 mL filtered sample.

12.3.3 Directly pour approximately 15-20 mL of sample into autosampler disposable test tubes.

12.4 Sample Preparation Procedures – Total Recoverable Direct Analysis for Drinking water samples with turbidity < 1 NTU:

12.4.1 Samples are preserved with 1 mL of HNO₃ for every 500 mL of sample in sample receiving area.

12.4.2 Check sample pH. Sample pH should be ≤ 2.

12.4.3 Directly pour approximately 10-15 mL of sample into autosampler disposable test tubes.

12.4.4 Type in sample sequence into computer system and run.

13. CALCULATIONS:

13.1 Instrument software automatically performs calculations such as dilution factors.

14. POLLUTION PREVENTION AND WASTE MANAGEMENT:

- 14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice.
- 14.2 Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation such as ordering smaller quantities of standards or preparing reagents in small amounts that can be used. The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations.
- 14.3 The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

15. CORRECTIVE ACTIONS FOR OUT OF CONTROL DATA:

- 15.1 See QA/QC Manager if QC Section requirements are out of tolerance.

16. REFERENCES:

- 16.1 Method for the Determination of Metals in Environmental Samples Supplement 1, EPA Method 200.7, Revision 4.4 May 1994.

17. TABLES, DIAGRAMS, FLOW CHARTS, Etc:

17.1 DLR Standard Preparation Table 1

Analyte	Initial Conc.	Amt. Used (mL)	DLR conc.	Final Vol. (mL)
Iron (Fe)	100 ppm	1.0	100 ppb	1000
Copper (Cu)	50 ppm	1.0	50 ppb	1000
Manganese (Mn)	20 ppm	1.0	20 ppb	1000
Zinc (Zn)	50 ppm	1.0	50 ppb	1000
Mn-LL	20 ppb	20	4 ppb	100
Sodium (Na)	1000 ppm	1	1 ppm	1000
Potassium (K)	1000 ppm	1	1 ppm	1000
Boron (B)	100 ppm	1	100 ppb	1000
Magnesium (Mg)	1000 ppm	1	1 ppm	1000
Aluminum (Al)	50 ppm	1	50 ppb	1000
Barium (Ba)	100 ppm	1	100 ppb	1000
Cobalt (Co)	10 ppm	1	10 ppb	1000
Molybdenum (Mo)	10 ppm	1	10 ppb	1000
Silica (SiO ₂)	1000 ppm	0.5	0.5 ppm	1000

Be, Cd	1.0 ppm	1.0	1.0 ppb	1000
Cr, Ni, Ag	1.0 ppm	1.0	10 ppb	100
Calcium (Ca)	1000 ppm	1	1 ppm	1000

17.2 LCS Standard Preparation Table 2

Analyte	Initial Conc.	Amt. Used (mL)	LCS conc.	Final Vol. (mL)
Iron (Fe)	500 ppm	5	2500 ppb	1000
Copper (Cu)	500 ppm	5	2500 ppb	1000
Manganese (Mn)	500 ppm	5	2500 ppb	1000
Zinc (Zn)	500 ppm	5	2500 ppb	1000
Mn-LL	20 ppb	50	10 ppb	100
Sodium (Na)	1000 ppm	50	50 ppm	1000
Potassium (K)	500 ppm	50	25 ppm	1000
Boron (B)	500 ppm	5	2500 ppb	1000
Magnesium (Mg)	500 ppm	50	25 ppm	1000
Aluminum (Al)	500 ppm	5	2500 ppb	1000
Barium (Ba)	100 ppm	5	500 ppb	1000
Cobalt (Co)	100 ppm	5	500 ppb	1000
Molybdenum (Mo)	100 ppm	5	500 ppb	1000
Silica (SiO ₂)	1000 ppm	25	25 ppm	1000
Be, Cd, Cr, Ni, Ag	50 ppm	5.0	500 ppb	500
Calcium (Ca)	500 ppm	50	25 ppm	1000

17.3 S-1 (High) Standard Preparation Table 3

Analyte	Initial Conc.	Amt. Used (mL)	S-1 conc.	Final Vol. (mL)
Iron (Fe)	500 ppm	10	5000 ppb	1000
Copper (Cu)	500 ppm	10	5000 ppb	1000
Manganese (Mn)	500 ppm	10	5000 ppb	1000
Zinc (Zn)	500 ppm	10	5000 ppb	1000
Mn-LL	1000 ppb	10	100 ppb	100
Sodium (Na)	1000 ppm	100	100 ppm	1000
Potassium (K)	500 ppm	100	50 ppm	1000
Boron (B)	500 ppm	10	1000 ppb	1000
Magnesium (Mg)	500 ppm	100	50 ppm	1000
Aluminum (Al)	500 ppm	10	1000 ppb	1000
Barium (Ba)	100 ppm	10	1000 ppb	1000
Cobalt (Co)	100 ppm	10	1000 ppb	1000
Molybdenum (Mo)	100 ppm	10	1000 ppb	1000
Silica (SiO ₂)	1000 ppm	50	50 ppm	1000
Be, Cd, Cr, Ni, Ag	50 ppm	10	1 ppm	500
Calcium (Ca)	1000 ppm	100	100 ppb	1000

17.4 IPC/IPCC Standard Preparation Table 4

Analyte	Initial	Amt. Used	IPC/ IPCC	Final Vol. (mL)
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	Conc.	(mL)	conc.	
Iron (Fe)	500 ppm	5	2500 ppb	1000
Copper (Cu)	500 ppm	5	2500 ppb	1000
Manganese (Mn)	500 ppm	5	2500 ppb	1000
Zinc (Zn)	500 ppm	5	2500 ppb	1000
Mn-LL	20 ppb	50	10 ppb	100
Sodium (Na)	1000 ppm	50	50 ppm	1000
Potassium (K)	500 ppm	50	25 ppm	1000
Boron (B)	500 ppm	5	2500 ppb	1000
Magnesium (Mg)	500 ppm	50	25 ppm	1000
Aluminum (Al)	500 ppm	5	2500 ppb	1000
Barium (Ba)	100 ppm	5	500 ppb	1000
Cobalt (Co)	100 ppm	5	500 ppb	1000
Molybdenum (Mo)	100 ppm	5	500 ppb	1000
Silica (SiO ₂)	1000 ppm	25	25 ppm	1000
Be, Cd, Cr, Ni, Ag	50 ppm	5	500 ppb	500
Calcium (Ca)	500 ppm	50	25 ppm	1000

17.5 Matrix Spike/Matrix Spike Duplicate Preparation Table 5

<i>Analyte</i>	<i>Initial Stock Conc. (mg/L)</i>	<i>Vol (μL) added in 25 mL of sample</i>	<i>Final Spike Conc. (mg/L)</i>
Be, Cd, Cr, Ni, Ag	20	625	0.5
Al, Ba, B, Co, Cu, Fe, Mn, Mo, Zn	100 & 500	125	0.5 & 2.5
Ca, Mg, Na, K	500 & 1000	1250	25 & 50
Si	1000	625	25

18. TRAINING AND QUALIFICATION VERIFICATION:

18.1 Signature of analyst and trainer on file.

19. HEALTH AND SAFETY:

19.1 The toxicity or carcinogenicity of each reagent used in the method has not been fully established. Each chemical should be regarded as a potential health hazard, and exposure to these compounds should be as low as reasonably achievable through using fume hoods.

19.2 Analytical plasma sources emit radio-frequency radiation of intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The ICP instrument has numerous "interlocks" which extinguish the plasma should hazardous conditions arise and these should never be defeated.

- 19.3 Precautions should also be taken to minimize other potential hazards. Basic good housekeeping and safety practices such as the use of rubber or plastic gloves, lab coat, and safety glasses are recommended.

Department: Inorganics	
Method Approved By:	
<div style="text-align: center;"><hr/><div>Khurshid Ahmed Department Supervisor</div><div>Date: _____</div></div>	
<div style="text-align: center;"><hr/><div>Bob Glaubig Laboratory Director</div><div>Date: _____</div></div>	<div style="text-align: center;"><hr/><div>Alex Popa QA/QC Manager</div><div>Date: _____</div></div>

CLINICAL LABORATORY OF SAN BERNARDINO, Inc.

**STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF AMMONIA BY
DISTILLATION AND COLORIMETRY**

1. SCOPE AND APPLICATION:

- 1.1 This method is applicable for surface, drinking, treated, waste and ground water.
- 1.2 The applicable range is 0.01-50 mg/L $\text{NH}_3\text{-N}$. Higher concentrations can be determined by sample dilution.

2. REPORTING LIMIT:

- 2.1 DLR is 0.5 mg/L for Ammonia as N and 0.6 mg/L for Ammonia as NH_3 .

3. APPLICABLE MATRIX AND MATRICES:

- 3.1 Please refer to Section 1.1.

4. SUMMARY OF THE METHOD:

- 4.1 A sample of water is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color is intensified with sodium nitroprusside and measure colorimetrically at 630 nm.

5. DEFINITIONS:

- 5.1 Batch – consists of twenty samples and must contain a LCS, MBLK, MS1, MS2, and a CCV every 10 samples.
- 5.2 Calibration Standard (CAL) – A series of standards prepared from the primary standard to calibrate tests.
- 5.3 Continuing Calibration Verification (CCV) – A midpoint primary source calibration standard run at the beginning and end of each analytical batch; used to verify the calibration.
- 5.4 Detection Limit for Reporting (DLR) – A practical limit that can be reported for the method which is usually 2 times higher or more than the MDL.
- 5.5 Flow Injection Analysis (FIA) – References to the Seal equipment.

- 5.6 Initial Calibration Verification (ICV) – A midpoint primary source calibration standard run at the end of every calibration; used to verify the calibration.
- 5.7 Laboratory Control Sample (LCS) – A control sample to check method performance
- 5.8 Linear Calibration Range (LCR) – The concentration over which the test is linear.
- 5.9 Matrix Spike (MS) – A sample that is spiked at mid-level concentration to check matrix effect of samples; it is also to determine precision and accuracy of this test.
- 5.10 Method Blank (MBLK) – Run every 20 samples used as a control sample to confirm a clean system free from interference.
- 5.11 Method Detection Limit (MDL) – Method Detection limit is at 99% confidence limit that the analyte concentration will be greater than zero.
- 5.12 Primary Dilution Standard (PDS) – A standard from a certified source used to calibrate test or develop a calibration curve.
- 5.13 Secondary Dilution Standard (SDS) – A standard from a certified source used to prepare the LCS, MS.

6. CONTAMINATION AND INTERFERENCES:

- 6.1 There can be potential contamination and interferences from using reagents that are not analytical grade. Potential interferences may come from glassware and containers.
- 6.2 Cyanate, which may be encountered in certain wastes, will hydrolyze to form ammonia.
- 6.3 Residual chlorine must be removed by pre-treatment with sodium thiosulfate.

7. APPARATUS AND MATERIALS:

- 7.1 Analytical Balance that weighs 0.0001 g.
- 7.2 100 mL Volumetric Flasks, Class A.
- 7.3 A Kontes Midi-Vap 2000 Distillation system (Model 47190-2000).
- 7.4 Auto Analyzer 3 High resolution.
- 7.5 2 channel manifold high resolution (HR) Digital Colorimeter.
- 7.6 Dell precision computer with 6.04 software and Dell 1720 Printer.
- 7.7 pH paper (for 9.5, use pH paper range 7-14 with 0.5 increments).

- 7.8 190 cm sample loop.
- 7.9 50 mL graduated cylinder.
- 7.10 180 Position auto-sampler.
- 7.11 Injection module.
- 7.12 13 x 100 mm glass culture tubes.

8. REAGENTS AND STANDARDS:

- 8.1 Reagent grade de-ionized water, Nanopure or equivalent.
- 8.2 Sodium Hydroxide 1 N: 40 g of Sodium Hydroxide in 1 Liter.
- 8.3 Borate buffer dissolve 9.5 of sodium borate decahydrate and 88 mL of 1 N NaOH in 1 Liter of water.
- 8.4 Boric Acid – 20 g of Boric Acid in 1 Liter of reagent water.
- 8.5 Sodium Phenolate: Mix 16.6 g of crystalline phenol and 6.4 g of NaOH with 100 mL of reagent water. Cool and make up to a final volume of 200 mL.
- 8.6 Sodium Hypochlorite: Add 26.25 mL of 6% hypochlorite solution and bring to up to 100 mL volume with reagent water.
- 8.7 Sodium Nitroprusside: Dissolve 32g of disodium EDTA and 0.4g of NaOH in about 600 ml of DI water. Then dissolve 0.18 g sodium nitroprusside and dilute to 1 liter with DI water. Mix thoroughly and add 3 ml of Brij-35 solution.

9. SAMPLE COLLECTION, PRESERVATION, SHIPMENT, AND STORAGE:

- 9.1 Samples are collected in plastic bottles. The holding time is 28 days.
- 9.2 Samples must be preserved with H_2SO_4 to a pH < 2 and cooled to $4 \pm 2^\circ\text{C}$.

10. QUALITY CONTROL:

- 10.1 Each batch is 20 samples and contains an LCS, MBLK. CCV and MS are run every 10 samples. Each run contains a DLR. MBLK must be less than 0.50 mg/L. The calibration curve correlation coefficient must be > 0.995.
- 10.2 The LCS concentration is 25 mg/L and must be within 90 – 110% acceptance. The MS concentration is 15 mg/L and must be within 90 – 110% acceptance.

11. CALIBRATION AND STANDARDIZATION:

- 11.1 Standardization is achieved using Auto Analyzer 3 High resolution 6.04 software.
- 11.2 All standard curves are linear and will run with a correlation coefficient of 0.995 or better for all ammonia analysis. Failure at this point will require a re-standardization or preparation of new standards.
- 11.3 All data stored with the Auto Analyzer 3 High resolution 6.04 software provides for imbedding of the calibration curve raw data. The electronic data file including the chromatograms can be retrieved and reviewed when needed.
- 11.4 Prepare calibration standards at concentrations of 0, 0.5, 5, 25, and 50 mg/L.
- 11.5 Load into auto-sampler after the instrument has properly warmed up and a stable baseline is obtained. Refer to Section 12 for instrument set-up.
- 11.6 Verify the calibration curve with an initial calibration verification (ICV) check. The ICV must be within 90 – 110% acceptance.
- 11.7 Use continuing calibration verification (CCV) standards at the beginning and end of every 10 samples to verify the calibration curve which must be within 90 – 110% acceptance.

12. PROCEDURE:

Part A Standard Distillation:

- 12.1 Set up the Midi-Vap 2000 Distillation Systems Colorimeter according to manufacturer's instructions. Connect part A and part B of the distillation flasks to the Midi-Vap 2000. Place anti-siphon glass on top of the distillation flask with the 125 mL Erlenmeyer flask on the bottom to collect the sample.
- 12.2 Ensure the Coolflow CFT-33 is filled with enough de-ionized water. Turn on the Coolflow and wait for the temperature to reach 4°C.
- 12.3 Turn on the Midi-Vap 2000 and set timer to 120 minutes with a temperature at 200°C.
- 12.4 Turn on condenser water and prepare a blank sample to clean out the system.
- 12.5 Prepare calibration standard and other QC standards as stated in Table 1.
- 12.6 Measure 50 mL of each standard into a graduated cylinder and transfer solution into the distillation flask. Add 2.5 mL of borate buffer.
- 12.7 Verify pH. The pH should be around 9.5. Adjust pH to 9.5 with 1 N NaOH if needed.
- 12.8 Quickly add boiling chips to each distillation flask.

- 12.9 In the Erlenmeyer flask add 5 mL of boric buffer.
- 12.10 The condenser tip must extend below the level of the boric buffer solution. Turn on Midi-Vap 2000 and distil for 120 minutes at 200°C. After distillation bring volume up to 50 mL.

Part B Sample Distillation:

- 12.11 Measure 50 mL each sample using a graduated cylinder and place into a distillation flask. Add 2.5 mL borate buffer to each sample.
- 12.12 Add 1 N Sodium Hydroxide solution as needed until the pH is 9.5.
- 12.13 Record the pH of the sample in the distillation logbook.
- 12.14 Pour solution into distillation flask and allow each sample to distillate.
- 12.15 Quickly add boiling chips to each distillation flask.
- 12.16 In the Erlenmeyer flask add 5 mL of 2% boric acid.
- 12.17 The condenser tip must extend below the level of the boric acid solution.
- 12.18 For MS preparation: Measure 50 mL of sample into a 50 mL volumetric flask. Add spike at 750 µL of SDS into volumetric flask and bring to volume with sample. Transfer solution into an Erlenmeyer flask. Add enough 1 N NaOH to bring pH to 9.5. Transfer solution into distillation flask. Repeat steps 12.13-12.17.
- 12.19 The temperature for ammonia distillation should be adjusted to 200°C for 121 minutes.
- 12.20 It takes approximately 10 minutes for distillation to reach equilibrium and requires approximately 2 hours total run time to recover 50 mL per station. After distillation bring each sample to a 50 mL volume.

Instrument Setup Procedure:

- 12.21 Ensure the 630 nm filters are in the colorimeter for NH₃-N method.
- 12.22 Place all feed lines into de-ionized water containing Brij-35 and switch on all Auto-analyzer 3 modules. The valve for the column must be in the closed position.
- 12.23 To start the pump, close the pump platen. Set the power switch located on the side of the pump to position 1, which is on. Set the red switch on top of the pump to position RUN and the black switch to position normal. The rollers will now start rotating.
- 12.24 Check bubble pattern in all lines, especially the flow cell waste line.

- 12.25 Check the Water baseline by starting charting option in AACE to display charts with the channel readings. The water baseline should be stable and flat.
- 12.26 Place the reagent lines from the wash receptacle into the reagent containers. Check that the bubble pattern is still okay with reagents; baseline should be stable and flat.
- 12.27 Select set-up and select analysis. In analysis select NH₃-N method. Select new run to enter the sample information. Select O.K. to save after all the samples have been entered. Name the sequence by the date started (year, month, day).
- 12.28 Load the standards and samples into the tubes according to sequence. Start the run, select the date of the run for the sequence and select O.K.
- 12.29 Check to see that LCS is running within the true value of +/- 10%. When the analysis is complete, remove the pump reagent lines and run reagent water through the system to clean the lines. If needed the lines can be cleaned with 1 N HCl and then rinsed with reagent water. Turn off the pump and modules. Release the pump tube cassettes.

13. CALCULATIONS:

- 13.1 Instrument software calculates results based on integration parameters.

14. CORRECTIVE ACTIONS FOR OUT OF CONTROL DATA:

- 14.1 Inform supervisor and notify QA/QC Manager for any non-conformance issues.

15. POLLUTION PREVENTION AND WASTE MANAGEMENT:

- 15.1 If samples are hazardous, dispose of after holding time in accordance with DOHS and EPA requirements.

16. REFERENCES:

- 16.1 EPA Method 350.1. "Determination of Ammonia Nitrogen by Semi-Automated Colorimetry." U.S. Environmental Protection Agency. (1993).
<<http://www.epa.gov/epahome/index/>>

- 16.2 Method MT G145-95 Rev.3. "Ammonia in Waters." Auto Analyzer 3

17. TABLES, DIAGRAMS, FLOW CHARTS, Etc:

- 17.1 Control Charts used to monitor performance.
- 17.2 Table 1: Standard Preparation

Standard Name	Initial Conc. (ppm)	Amt. Added (mL)	Final Conc. (ppm)	Final Volume (mL)
---------------	---------------------	-----------------	-------------------	-------------------

Cal-1	1000	25	50	500
Cal-2	1000	25	25	1000
Cal-3	25 (PDS)	100	5.0	500
Cal-4	25 (PDS)	10	0.5	500
Cal-5	0	0	0.0	500
DLR*	1000	0.5	0.5	1000
LCS/*	1000	25	25	1000
MS*	1000	15	15	1000
ICV/ CCV	1000	25	25	1000

*Uses a secondary source standard (SDS) for preparation.

18. TRAINING and QUALIFICATION VERIFICATION:

18.1 Signature of analyst and trainer on file with qualifying card.

19. HEALTH and SAFETY:

19.1 Proper safety procedures should be used for handling chemicals used in this test. The method analytes used in this test are classified as hazardous and appropriate safety procedures implemented such as respirator, lab coat, rubber gloves and glasses.

19.3 MSDS on file for chemical reference as needed.

Department: Inorganics	
Method Approved By:	
<div style="text-align: center; margin-bottom: 20px;"> <hr style="width: 60%; margin: 0 auto;"/> <p>Khurshid Ahmed Department Supervisor</p> </div> <div style="text-align: center; margin-bottom: 20px;"> <p>Date: <hr style="width: 200px; display: inline-block; vertical-align: middle;"/></p> </div> <div style="display: flex; justify-content: space-between; margin-bottom: 20px;"> <div style="text-align: center; width: 45%;"> <hr style="width: 80%; margin: 0 auto;"/> <p>Bob Glaubig Laboratory Director</p> </div> <div style="text-align: center; width: 45%;"> <hr style="width: 80%; margin: 0 auto;"/> <p>Alex Popa QA/QC Manager</p> </div> </div> <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>Date: <hr style="width: 150px; display: inline-block; vertical-align: middle;"/></p> </div> <div style="width: 45%;"> <p>Date: <hr style="width: 150px; display: inline-block; vertical-align: middle;"/></p> </div> </div>	

Clinical Laboratory of San Bernardino, Inc.

Standard Operating Procedure for the Determination of Anionic Surfactant as MBAS

1. SCOPE AND APPLICATION:

- 1.1 This method applies to ground water, drinking water and selected wastewaters, for determining the anionic surfactants in water samples down to 0.1 mg/L.

2. REPORTING LIMIT:

- 2.1 The detection limit for reporting DLR is 0.1 mg/L, the MDL is 0.047 mg/L (March 2014).

3. APPLICABLE MATRIX AND MATRICES:

- 3.1 Please refer to Section 1.1.

4. SUMMARY OF METHOD:

- 4.1 Methylene Blue Active substances (MBAS) transfer methylene blue, a cationic dye, from an aqueous solution into an immiscible organic liquid (chloroform) upon equilibration. The intensity of the resulting blue color in the organic phase is a measure of MBAS. This method is relatively simple and precise. It comprises of three successive extractions from acid aqueous medium containing excess methylene blue into chloroform, followed by an aqueous backwash and measurement of the blue color in the chloroform by spectrophotometry at 652 nm. The samples are compared to a standard curve and results calculated. Sample results are reported referencing molecular weight of the LAS standard; currently 340.0 g/mol.

5. DEFINITIONS:

- 5.1 Batch – consists of twenty samples and contains an LCS, LCSD, MBLK, DLR, MS, MSD and an opening and closing CCV.
- 5.2 Calibration Standard (CAL) – A solution prepared from a LAS stock standard to be used for instrument calibrations.
- 5.3 Continuing Calibration Verification (CCV) – A midpoint primary source calibration standard run at the beginning and end of an analytical batch.
- 5.4 Detection Limit for Reporting (DLR) – Typically the low level limit set by the state that is not laboratory specific (since the DLR was not specified, we report MBAS as 0.1 mg/L).

- 5.5 Initial Calibration Verification (ICV) – A midpoint primary source calibration standard run at the end of every calibration.
- 5.6 Laboratory Control Sample (LCS) – An aliquot of reagent water spiked with stock LAS standard used to confirm method calibration.
- 5.7 Linear Alkyl-benzene sulfonate (LAS) – Anionic Stock Standard purchased from manufacturer used as a spike for method calibrations, DLR check, LCS, MS, and MSD.
- 5.8 Matrix Spike (MS) and Matrix Spike Duplicate (MSD) – Randomly selected samples that are spiked with a LAS standard used to determine matrix effect.
- 5.9 Method Blank (MBLK) – A sample of reagent water used to check system cleanliness.
- 5.10 Methylene Blue Active Substances (MBAS) – A Methylene Blue Cationic Dye is optically active with anionic standards and forms a blue colorant.
- 5.11 Quality Control Sample (QCS) – A chosen random sample used for the MS and MSD.

6. CONTAMINATION AND INTERFERENCES:

- 6.1 The samples may contain a variety of colorants active with Methylene Blue dye that will cause interferences. The more insoluble the colorant, the more it will be removed by washing of the chloroform.
- 6.2 Other cationic substances can interfere with the test by forming ion pairs with the anions from competition.
- 6.3 Sulfides are interferences and can be removed from the sample by prior oxidation with hydrogen peroxide.

7. APPARATUS AND MATERIALS:

- 7.1 A spectrophotometer that can measure colorants at a 652 nm wavelength, providing a path length of 1 cm or greater.
- 7.2 500 mL glass separatory funnels with TFE stoppers and stopcocks.
- 7.3 Glass wool.
- 7.4 100 mL volumetric flask.
- 7.5 125 mL Erlenmeyer graduated flask.
- 7.6 200 mL graduated metric cylinder.

7.7 Glass funnels.

8. REAGENTS AND STANDARDS:

- 8.1 LAS Stock Solution – A standard solution is purchased from VHG #2300856 which is approximately 1000 mg/L concentration.
- 8.2 LAS Standard Solution – Dilute 10 mL of stock to 1 liter, which gives approximately 10 mg/L Working Standard Solution. Prepare daily.
- 8.3 Alcoholic Phenolphthalein Indicator.
- 8.4 1 N Sodium Hydroxide, (NaOH).
- 8.5 1 N and 6 N Sulfuric Acid, (H₂SO₄), solution.
- 8.6 Chloroform, reagent grade solvent.
- 8.7 Methylene Blue Reagent (MB) – weigh 100 mg of MB in 100 mL of water; transfer 30 mL to a 1000 mL flask. Add 500 mL water, 41 mL of 6 N sulfuric acid, and 50 g of NaH₂PO₄-H₂O and shake and dissolve.
- 8.8 MBAS Wash Solution – Add 123 mL of 6 N sulfuric acid, 150 g of NaH₂PO₄-H₂O, and dilute to 3 liter with reagent water.
- 8.9 Methanol, reagent grade solvent.
- 8.10 30% Hydrogen Peroxide, (H₂O₂).
- 8.11 Water, reagent grade, MBAS free de-ionized water.

9. SAMPLE COLLECTION, PRESERVATION, SHIPMENT, AND STORAGE:

- 9.1 200 mL samples will be sufficient to run most samples.
- 9.2 The hold time for analysis is 48 hours.

10. QUALITY CONTROL:

- 10.1 A batch consists of 20 samples plus QC checks.
- 10.2 QC checks per batch include LCS, LCSD, MBLK, DLR, MS, MSD, CCV.
- 10.3 LCS is run at the beginning of the batch and at the end of the batch of 20 samples.
- 10.4 DLR checks, MS, and MSD are run once per batch of 20 samples.

- 10.5 Record all stock standards purchased and all working standards prepared in the standard inventory log and the standard prep log respectively.
- 10.6 All standards used in the method must be properly labeled with date received, date opened, and date expired along with respective analyst's initials.
- 10.7 Record any deviations and non-conformances in the bound corrective action logbook. All QC checks and sample results are reported in the bound run log book.
- 10.8 DLR checks must be within 75% to 125% recovery.
- 10.9 Beginning and ending CCV, LCS, and LCSD must be within 90 to 110% recovery.
- 10.10 MS and MSD must be within 80 to 120% recovery.
- 10.11 MBLK value must be less than 0.1 mg/L at all times.
- 10.12 LCS and Matrix Spike RPD limits must be less than 20%.

11. CALIBRATION AND STANDARDIZATION:

- 11.1 A five point calibration is prepared monthly with the purchased stock standard. Refer to Table 1 in Section 17.
- 11.2 Set the HACH 2800 Spectrophotometer to a 652 nm wavelength.
- 11.3 Use sterile 13 x 100 mm disposable culture tubes and pour sample filling to the top of the culture tube.
- 11.4 Place the blank sample in the 1 cm cell and zero the instrument by pushing zero on the display.
- 11.5 Ensure the linear regression correlation of coefficient (r) is ≥ 0.995 .
- 11.6 Verify the calibration with continuous QC check samples at the beginning and end of 20 samples.

12. PROCEDURE:

- 12.1 Calibration Curve, QC checks, and samples are extracted simultaneously.
- 12.2 Clean 500 mL separatory funnels and ensure no leakage occurs.
- 12.3 Prepare a 0.0 calibration standard by adding 200 mL of de-ionized water into a 500 mL separatory funnel.

- 12.4 Prepare a 0.1 calibration standard by adding 198 mL of de-ionized water spike with 2 mL of standard and place in a 500 mL separatory funnel.
- 12.5 Prepare a 0.5 calibration standard by adding 190 mL of de-ionized water spiked with 10 mL of standard and place in a 500 mL separatory funnel.
- 12.6 Prepare a 1.0 calibration standard, ICV, and CCV by adding 180 mL of de-ionized water spiked with 20 mL of standard and place in a 500 mL separatory funnel.
- 12.7 Prepare a 2.0 calibration standard by adding 160 mL of de-ionized water spike with 40 mL of standard and place in a 500 mL separatory funnel.
- 12.8 Prepare a MBLK by adding 200 mL of de-ionized water into a 500 mL separatory funnel.
- 12.9 Prepare a DLR check by adding 200 mL of de-ionized water spiked with 20 mL of standard into a 500 mL separatory funnel.
- 12.10 Prepare an LCS and LCSD by adding 196 mL of de-ionized water spike with 4 mL of LAS standard into a 500 mL separatory funnel.
- 12.11 Prepare an MS and MSD by adding 100 mL of randomly chosen sample spiked with 2 mL of LAS standard to each 500 mL separatory funnel respectively.

NOTE: To avoid dis-coloration of methylene blue sulfides, add enough peroxide to sample for treatment until color changes to blue.
- 12.12 Add 1 drop of phenolphthalein indicator to each separatory funnel.
- 12.13 Add dropwise 1 N NaOH to each separatory funnel, until color changes to pink.
- 12.14 Add 1 N H₂SO₄, drop wise, until pink color disappears.
- 12.15 Add 25 mL of methylene blue reagent and 10 mL of chloroform to each separatory funnel.
- 12.16 Shake, vent, and rock each separatory funnel vigorously for 30 seconds and allow standing so the layers can separate.
- 12.17 Drain the chloroform layers into a 125 mL pre-cleaned Erlenmeyer flask.
- 12.18 Repeat with 10 mL of chloroform to each separatory funnel.
- 12.19 Repeat steps 12.16-12.18 for extraction two more times.
- 12.20 Pour out water to clean and re-use separatory funnel.

- 12.21 Combine all resulting chloroform extracts in 125 mL Erlenmeyer flask and pour into its respective separatory funnel.
- 12.22 Add 50 mL of MBAS wash solution into each separatory funnel.
- 12.23 Shake, vent, and shake again each separatory funnel vigorously for 30 seconds and allow standing so the layers can separate.
- 12.24 Set-up glass funnels with a plug of glass wool to filter extract.
- 12.25 Drain the chloroform layer into a pre-cleaned 100 mL volumetric flask through a glass wool plug.
- 12.26 Repeat steps 12.23 through 12.25 two more times by adding 10 ml of chloroform and then place in the 100 mL volumetric flask. Rinse glass wool and funnel with chloroform. Collect washings in the volumetric flask.
- 12.27 The aqueous phase can be discarded after the final extraction.
- 12.28 Bring volume up to 100 mL with chloroform.
- 12.29 If sulfide is known to be present add a few drops of hydrogen peroxide to the chloroform layer.
- 12.30 After calibration of spectrophotometer, rinse culture tubes with samples and read all extracts.
- 12.31 Record all calibration standards, reference numbers, QC readings, and samples in the run logbook.

13. CALCULATIONS:

- 13.1 Calculate recovery of LCS: $\frac{\text{Conc (mg/L)} \times 100}{\text{Spike (mg/L)}} = \% \text{ Recovery}$
- 13.2 Recovery (MS): $\frac{\text{Conc} - \text{Sample Result} \times 100}{\text{Spike (mg/L)}} = \% \text{ Recovery}$
- 13.3 Relative Percent Difference (RPD): $\% \text{ RPD} = \frac{|S1 - S2|}{(S1 + S2)/2} \times 100$

14. POLLUTION PREVENTION AND WASTE MANAGEMENT:

- 14.1 Discharge samples and solvents according to State Regulations.

15. CORRECTIVE ACTIONS FOR OUT OF CONTROL DATA:

15.1 See QA/QC Manager for proper documentation for any non-conformance related issues.

16. REFERENCES:

16.1 Standard Methods for the Examination of Water and Wastewater. 20th Edition, Section 5-47. Method 5540C. "MBAS" (1998).

17. TABLES, DIAGRAMS, FLOWCHARTS, Etc:

17.1 Table 1: Calibration Chart

Standard Name	Initial Conc. (mg/L)	Amt. Used (mL/uL)	Final Conc. (mg/L)	Final Vol. (mL)
Cal-1	10	0.0	0.0	200
Cal-2	10	2.0	0.10	200
Cal-3	10	10	0.50	200
Cal-4/ICV/CCV	10	20	1.0	200
Cal-5	10	40	2.0	200
LCS/LCSD	60	4.0	1.2	200
DLR	10	2.0	0.10	200
MS/MSD	60	2.0	1.2	100

18. TRAINING AND QUALIFICATION VERIFICATION:

18.1 Signature of analyst and trainer on file.

19. HEALTH AND SAFETY:

19.1 Chloroform is a suspected carcinogen, so contact with the skin or breathing should be avoided. Refer to MSDS on file for proper chemical handling and safety procedures.

19.2 Protective eyewear, lab coats, close-toed shoes, and safety gloves must be worn at all times while running this test. The extraction has to be done under the hood. The rack of separatory funnels must be in the hood. The spectrophotometer must be in the hood also while reading the absorbance.

Department: Inorganics

Method Approved By:

Khurshid Ahmed
Department Supervisor

Date: _____

Bob Glaubig
Laboratory Director

Date: _____

Alex Popa
QA/QC Manager

Date: _____

APPENDIX D

BABCOCK LABORATORIES, INC. STANDARD OPERATING PROCEDURES

Babcock Laboratories, Inc.
STANDARD OPERATING PROCEDURE
COLIFORMS BY COLILERT® P/A (MMO-MUG,UV)
SM 9223B

Effective Date 12/5/2014

1.0 Scope and Application

- 1.1 Chromogenic substrates, such as ortho-nitrophenyl- β -D-galactopyranoside (ONPG) are used to detect enzyme β -D-galactopyranoside, which is produced by total coliform bacteria. The β -D-galactopyranoside enzyme hydrolyzes the substrate and produces a color change after incubation.
- 1.2 This method is used for drinking water and surface water samples.

2.0 Summary of Method

- 2.1 A Colilert reagent packet is added to 100 mL of sample. Sample is incubated. If the sample is clear after incubation, coliform is absent from the sample. If the sample is as yellow as or more yellow than the Colilert® comparator, the sample is considered present for coliform bacteria. The sample is placed under a 365 nm fluorescent light. If the sample fluoresces it is considered present for *Escherichia coli*.

3.0 Definitions: See SOP Q15 – SOP Definitions

4.0 Interferences

- 4.1 Water samples containing humic or other material may be colored. If there is background color, compare inoculated bottle to a control bottle containing only the water sample or analyze sample by Multiple Tube Fermentation.
- 4.2 In certain waters, high calcium salt content can cause precipitation but this should not affect the reaction.
- 4.3 The presence of chlorine is detrimental to bacterial growth. Sodium thiosulfate is added to all sample bottles to treat for residual chlorine. If the sodium thiosulfate in the sample bottle is not sufficient to treat for the residual chlorine contained in the sample, the sample will turn blue after addition of Colilert. In this event results are qualified with:

N_BCI: Results are suspect due to excess chlorine residual present at time of test.

5.0 Safety

- 5.1 When dealing with bacteriological testing it is important to ensure that all working surfaces are clean. Gloves, safety glasses and lab coats are recommended to protect the analyst from exposure to bacteria.

6.0 Equipment and Supplies

- 6.1 Glass Pipets: 10 mL
 - 6.1.1 Pipets are placed in disinfectant and then washed thoroughly with D.I. water and then sterilized in a drying oven overnight.
- 6.2 Incubators ($35^{\circ} \pm 0.5^{\circ}\text{C}$)
- 6.3 365 nm Fluorescent Light
- 6.4 Water bath (44.5°C)

7.0 Reagents and Standards

- 7.1 Colilert® reagent packets stored at room temperature until manufacturer expiration date.
- 7.2 Dilution water bottles – See ESB SOP B01 section 6.0
- 7.3 Reagent water – Nanopure
- 7.4 Our laboratory uses reference cultures of organisms obtained from ATCC, (American Type Culture Collection). The reference cultures are stored at 4°C until manufacturer expiration date. Reference cultures are rehydrated in Lauryl Sulfate Broth at $35^{\circ} \pm 0.5^{\circ}\text{C}$ for 15 minutes or until dissolved. A loop full of the suspension is streaked onto a Standard Methods agar slant and kept at $35^{\circ} \pm 0.5^{\circ}\text{C}$ for up to a month. The slant is used to prepare a working plate on Tryptic Soy Agar which is also kept at $35^{\circ} \pm 0.5^{\circ}\text{C}$. New plates are prepared from the slant as needed.
 - 7.4.1 *Enterobacter aerogenes*- coliform (not E. coli): yellow, nonfluorescent
 - 7.4.2 *Pseudomonas aeruginosa* – negative: noncoliform
 - 7.4.3 *Escherichia coli* – positive: yellow and fluoresces

8.0 Sample Collection, Preservation and Storage

- 8.1 Samples are collected in sterile 125 mL containers containing $\text{Na}_2\text{S}_2\text{O}_3$ for chlorine removal.
- 8.2 Samples must be kept cold, a suggested $<10^{\circ}\text{C}$ for drinking waters from sampling until analysis. If a sample arrives on ice, hand delivered and sampled on the arrival date, it is assumed that the cooling process has begun.
- 8.3 Samples must be analyzed within 30 hours for drinking waters.

Note: Sampled date and time for bottled water samples are documented as the date and time the sample was received by the laboratory.

9.0 Procedure

Note: Thoroughly agitate sample to ensure homogeneity prior to withdrawal of aliquot. If no head space is observed, transfer the sample to a larger sterile container and agitate the sample to ensure homogeneity. Transfer approximately 110 mL back to its original container.

- 9.1 Upon receiving the sample, one milliliter is pipetted into each of two sterile petri dishes when needed, for the Heterotrophic Plate Count analysis. (Heterotrophic Plate Count SOP B07 for procedure.)
- 9.2 A sterile pipette is used to remove sample from the vessel to adjust the volume to 100 milliliters.

Note: If the sample requires dilution, the appropriate aliquot of sample is diluted into 100 mL of sterile D.I. water.

- 9.3 One unit of Colilert® is added to the vessel. Thoroughly agitate the sample to ensure proper mixing and then incubated $35^{\circ} \pm 0.5^{\circ}\text{C}$.
 - 9.3.1 Samples received before noon may be analyzed using 24hr Colilert® and incubated for 24-28 hours.
 - 9.3.2 Generally most samples will be analyzed using 18hr Colilert® and incubated for 18-22 hours.
 - 9.3.2.1 When used for P/A analysis, 18hr Colilert® requires a pre-heating step:
 - 9.3.2.1.1 After the 18 hour Colilert® has been added, the P/A vessel is warmed in a designated 44.5°C water bath for 7-10 minutes (A timer is set for 10 minutes).
 - 9.3.2.1.2 The time the vessel is placed in the bath and the time the vessel should be removed from the bath is noted on the vessel prior to setting it in the bath. This will ensure that the vessel is not kept in the bath for more than 10 minutes.
- 9.4 If the sample is clear after its incubation period, coliform is absent from the sample. If the sample is as yellow as or more yellow than the Colilert® comparator at 22 hours, the sample is considered present for coliform bacteria. If the sample fluoresces, it is considered present for *Escherichia coli*. When fluorescent results are uncertain, samples may be taken to a dark room for reading.
 - 9.4.1 When using 24 hour Colilert®, if the sample is a paler shade of yellow than the comparator after 24 hours of incubation, the sample is allowed to incubate for an additional 4 hours. If no color change is noted, the sample is considered absent for coliform. If the sample has become as yellow as or more yellow than the comparator, the sample is now considered present for coliform. If the sample fluoresces in 365 nm light, the sample is considered present for *Escherichia coli*. When fluorescent results are uncertain, samples may be taken to a dark room for reading.
 - 9.4.2 Samples that turn turbid without any yellow color during Colilert® analysis must be invalidated. A note indicating turbidity is placed on the bottom of the lab sheet.
- 9.5 Quanti-Tray®: Upon request samples may be analyzed by Quanti-Tray®. A sterile pipette is used to remove sample from the vessel to adjust the volume to 100 mL. One unit of Colilert® is added to the vessel. The vessel thoroughly agitated to ensure proper mixing. Once the media is completely dissolved, it is poured into the Quanti-Tray®. Get rid of air bubbles, seal and incubate for 24-28 hours at $35^{\circ} \pm 0.5^{\circ}\text{C}$. If any wells are yellow after incubation, they are positive for coliform. Fluorescing wells

are considered positive for *E. coli*. Depending on the number of positive wells, an MPN can be recorded. The MPN is calculated from the MPN chart supplied by the manufacturer. The reporting limit is 1.0 MPN/100 mL.

9.5.1 Colilert® 18hr Quanti-Trays® do not require preheating. Samples are incubated for 18-22 hours at $35^{\circ} \pm 0.5^{\circ}\text{C}$.

- 9.6 In order to prevent incorrect client notification of positive results, it is imperative that the analyst checks to make sure the label on the positive sample bottle matches the identification of the sample lab sheet. In addition, peer reviewing and documentation is required. Monday thru Friday when a sample is positive for coliform using Colilert®, (P/A, Quanti-Tray®, or 10 tube), peer reviewing must take place prior to client notification. This involves an additional analyst double checking the recorded results and making sure the correct sample on the lab sheet has been marked positive. The peer-review analyst records their initials and the date on the right hand side of the lab sheet next to the positive result. On the weekends, after client notification, the analyst must leave their positive samples on the counter where the Colilert® readings took place. The analyst working the next business day must recheck, initial and date the recorded results.

10.0 Quality Control:

Note: See also ESB SOP B01 for general QC requirements

- 10.1 Whenever Colilert® is used to detect coliform bacteria, a batch of culture controls must be incubated along with the samples. These cultures and a blank are used to ensure reproducible results and to check the quality of the Colilert® and incubation temperature. A unit of Colilert® is added to a dilution bottle containing 90 mL sterile Nanopure water. After the Colilert® is dissolved, 10-20 mL portions are pipetted into four sterile test tubes. The tubes are individually labeled and inoculated with *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. The fourth tube is labeled as a blank. These four tubes are placed in the incubator designated to incubate that day's Colilert® samples. After the appropriate incubation time, QC results are recorded in the QC notebook along with the Colilert® lot number and expiration date.

10.2 Duplicates:

- 10.2.1 Sample duplicates are performed at a frequency of 5%.
10.2.2 If sample duplicate results do not agree, see ESB SOP B08 section 7.0 for instructions.

11.0 Method Performance

- 11.1 Refer to in-house quality control performance records.

12.0 Corrective Action For Out of Control Or Unacceptable Data:

- 12.1 If any of the above QC samples do not meet acceptance criteria, the analyst must take measures to correct the problem.
12.1.1 The analyst examines the results of other controls performed that day.
12.1.2 The analyst examines sample results either for historical trends or for none detects.

- 12.1.3 If the above investigation indicates that the batch is still valid and the out of control QC appears to be an anomaly, results are reported.
- 12.1.4 If it is unclear whether sample results can be verified, any affected samples must either be resampled or results must be reported with a note qualifying the data.
- 12.1.5 Out of control QC is noted on the QC data page along with the corrective action taken. A QC follow up form is filled out in the computer and added behind the QC data page.

13.0 Pollution Prevention and Waste Management

- 13.1 All positive samples and QC are autoclaved prior to disposal.
- 13.2 See also SOP S07 – Pollution Prevention

Method Reference: Standard Methods For the Examination of Water and Wastewater
APHA, AWWA, WEF 22nd Edition Method 9223B.

Note: All *italicized items* (except bacteria names) are an indication of a variation from the method.

Approved by Stacey A. Fry Date 11/25/2014

BABCOCK LABORATORIES, INC. STANDARD OPERATING PROCEDURE
COLIFORMS BY
MULTIPLE TUBE FERMENTATION
SM 9221 B,C,E & AOAC 966.24
Effective Date 10/25/13

1.0 Scope and Application

- 1.1 The coliform group consists of several genera of bacteria belonging to the family *Enterobacteriaceae*. This group is defined as all aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h at 35°C.
- 1.2 Results of the examination of replicate tubes are reported in terms of Most Probable Number (MPN) per 100 mL of sample. This number, based on certain probability formulas, is an estimate of the mean density of coliforms in the sample. Coliform density, together with other information obtained by engineering or sanitary surveys, provides the best assessment of water treatment effectiveness and the sanitary quality of untreated water.
- 1.3 This method is used for drinking water, surface water, source water, wastewater samples, and solids.
- 1.4 Reporting limit: 2 MPN/100 mL (1.1 MPN/100 mL for ten tube)
- 1.5 Range: 2 – 1600 MPN/100 mL

2.0 Summary of Method

- 2.1 Sample is placed at various dilutions into tubes containing Lauryl sulfate broth and incubated for up to 48 hours, at 35°C ± 0.5°C. Each broth tube which shows CO₂ formation inside the Durham tube is transferred to a brilliant green bile tube. These tubes are incubated at 35°C for a total of 48 hours. If gas formation is present in their Durham tubes the sample is considered positive for total coliform bacteria. The positive broth tubes are also transferred to E.C. media (wastewaters and source waters), or E.C. + MUG (drinking waters). The tubes are incubated at 44.5°C ± 0.2°C for 24 ± 2 hours in the fecal water bath. If CO₂ formation is observed in the EC tube after the incubation period, the sample is considered positive for fecal coliform bacteria. If CO₂ formation is observed in the EC + MUG tube and the tube fluoresces in UV light, the sample is considered positive for *E. coli*.

3.0 Definitions: See SOP Q15 – SOP Definitions

4.0 Interferences

- 4.1 If the sample is not adequately shaken before portions are removed or if clumping of bacterial cells occurs, the MPN value will be an underestimate of the actual bacterial density.
- 4.2 The presence of chlorine is detrimental to bacterial growth. Sodium thiosulfate is added to all sample bottles to treat for residual chlorine.
- 4.3 Turbidity: See sections 11.1 and 11.2.

- 4.4 An arbitrary 48 hour limit for observation doubtless excludes occasional members of the coliform group that grow very slowly.
- 4.5 Even when five fermentation tubes are used, the precision of the results obtained is not of a high order. Consequently, exercise great caution when interpreting the sanitary significance of coliform results obtained from the use of a few tubes with each sample dilution, especially when the number of samples from a given point is limited.

5.0 Safety

- 5.1 When dealing with bacteriological testing it is important to ensure that all working surfaces are clean. Gloves are recommended to protect the analyst from exposure to bacteria.

6.0 Equipment and Supplies

- 6.1 Media tubes with Durham tube inserts (see ESB SOP B01 section 6.0 for washing instructions.)
- 6.2 Metal racks
- 6.3 Glass Pipets: 10 mL
 - 6.3.1 Pipets are placed in disinfectant and then washed thoroughly with D.I. water and then sterilized in a drying oven overnight.
- 6.4 Transfer loops - sterilized in a drying oven overnight
- 6.5 Labels
- 6.6 365 nm Fluorescent light
- 6.7 Water bath at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$
- 6.8 Incubators at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$

7.0 Reagents and Standards

- 7.1 Lauryl Sulfate Broth, Brilliant Green Bile, EC Media, and EC + MUG.
 - 7.1.1 Weekly Preparation:
 - 7.1.1.1 Directions on media labels are followed for proper rehydration.
 - 7.1.1.1.1 LSB: 356g media for every 5 liters of Nanopure.
 - 7.1.1.1.2 BGB: 120g media for every 3 liters of Nanopure
 - 7.1.1.1.3 EC: 111g media for every 3 liters of Nanopure
 - 7.1.1.1.4 EC + MUG: 111g media for every 3 liters of Nanopure
 - 7.1.1.2 Lauryl Sulfate Broth is rehydrated at double strength with Nanopure water as indicated in Standard Methods when used with 10 mL aliquots of sample. 12 mL of the rehydrated medium (so that the final volume is 10 mL after sterilization) are added to each test tube along with an inverted Durham tube which sits inside the medium tube. A loosely fitting lid is then added to each test tube before it is autoclaved.
 - 7.1.1.3 Prepared media is autoclaved for 12-15 minutes at 250°F (121°C) and 15 lb/in² pressure.
 - 7.1.1.4 All sterilized media is stored for later use in a cool cabinet out of direct sunlight, with loose fitting caps, for up to two weeks. A previous study showed that evaporation of media

does not exceed 1 mL until 3 months have elapsed. Each rack of media is identified and dated and rotated to ensure the oldest media is used first. Tubes stored longer than two weeks must have either a foam plug or screw cap

- 7.1.2 The pH of media is recorded after autoclaving. One tube from each batch of media made is autoclaved in a beaker along with the prepared media. The tube is allowed to cool to room temperature before the pH is taken. If pH is outside the following acceptance criteria new media is prepared.
- 7.1.2.1 Lauryl Sulfate Broth: 6.8 ± 0.2
 - 7.1.2.2 Brilliant Green Bile: 7.2 ± 0.2
 - 7.1.2.3 EC Media and EC + MUG: 6.9 ± 0.2
- 7.1.3 Each batch of media prepared is tested with a positive and negative control.
- 7.1.3.1 When a batch of media is prepared, four tubes are designated as QC tubes.
 - 7.1.3.2 After being autoclaved, one tube is inoculated with *Escherichia coli*, one tube with *Enterobacter aerogenes*, one tube with *Pseudomonas aeruginosa*, and the last tube is designated as a blank.
 - 7.1.3.3 These tubes are incubated for 48 hours at $35.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. (EC and EC + MUG are incubated at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ for 24 hours.) Results are recorded in the Bacteriology QC log book.
- 7.2 Eosin methylene blue agar (SM 15th Ed.)– 18.8g media for every 500 mL of Nanopure. Final pH must be 7.1 ± 0.2 . See ESB SOP B01 sections 5.2 and 5.3 for agar preparation instructions.
- 7.3 Dilution water bottles – See ESB SOP B01 section 5.0
- 7.4 Reagent water - Nanopure
- 7.5 Our laboratory uses reference cultures of organisms obtained from ATCC, (American Type Culture Collection). The reference cultures are stored at 4°C until manufacturer expiration date. Reference cultures are rehydrated in Lauryl Sulfate Broth at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 15 minutes or until dissolved. A loop full of the suspension is streaked onto a Standard Methods Agar slant and kept at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for up to a month. The slant is used to prepare a working plate on Tryptic Soy Agar which is also kept at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. New plates are prepared from the slant as needed.

	LSB	BGB	EC	EC+MUG	EMB
<i>Enterobacter aerogenes</i>	+	+	-	-	N/A
<i>Pseudomonas aeruginosa</i>	-	-	-	-	N/A
<i>Escherichia coli</i>	+	+	+	+	+
<i>Enterococcus faecalis</i>	N/A	N/A	N/A	N/A	partial

8.0 Sample Collection, Preservation and Storage

- 8.1 Samples are collected in sterile 120 mL containers containing $\text{Na}_2\text{S}_2\text{O}_3$ for chlorine removal.
- 8.2 Samples must be kept cold from sampling until analysis, $< 10^{\circ}\text{C}$ for wastewaters and a suggested $< 10^{\circ}\text{C}$ for drinking waters. If a sample arrives on ice, hand delivered and sampled on the arrival date, it is assumed that the cooling process

has begun. If a wastewater sample arrives $\geq 10^{\circ}\text{C}$ without ice, the client is advised that the sample temperature may affect test results.

- 8.3 Samples must be analyzed within 30 hours for drinking water, 8 hours for liquids and 24 hours for sludges.

9.0 Inoculation of Drinking Water Samples:

Note: Thoroughly agitate sample to ensure homogeneity prior to withdrawal of aliquot.

- 9.1 10 mL of sample is transferred to each of 10 tubes containing 10 mL of double strength sterile broth.
- 9.2 All broth tubes are placed in incubators. The broth tubes are allowed to incubate for 24 ± 2 hours, examined, and then incubated for an additional 24 ± 3 hours, if needed at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.
- 9.3 Note: Heterotrophic plate counts are also set up at this time. (see SOP B07).

10.0 Inoculation of non-drinking waters such as effluents, stormwaters, source waters and other surface waters and solids.

Note: Thoroughly agitate sample to ensure homogeneity prior to withdrawal of aliquot.

10.1 Liquids and semi-solids:

- 10.1.1 Generally a multiple dilution or serial dilution is used on non-drinking water which may contain high amounts of bacteria. A series of dilutions are needed in order to calculate the most probable number of bacteria per 100 mL (MPN/100mL).
- 10.1.1.1 Five 10 mL portions of sample are inoculated into the first 5 broth tubes.
- 10.1.1.2 10 mL of sample is then transferred into 90 mL of sterile dilution water. This dilution represents 1 mL of sample per 10 mL aliquot of solution. Five 10 mL portions of this first dilution are inoculated into the following five broth tubes.
- 10.1.1.3 10 mL of the first dilution are transferred into 90 mL of sterile dilution water. This second dilution represents 0.1 mL of sample per 10 mL aliquot of solution. Five 10 mL portions of this second dilution are inoculated into the third set of five broth tubes.
- 10.1.2 If the sample is cloudy or contains a small amount of dirt, a third dilution may be considered. If the sample is very dirty, additional dilutions will be needed. Raw liquid influents are generally diluted to 1×10^{-3} mL at the beginning of the series and ending at 1×10^{-6} mL. Semi-solid raw influents usually are started at 1×10^{-5} mL and end at 1×10^{-8} mL. 99.0 mL and 99.9 mL dilution bottles may be used for these higher dilutions.

10.2 Solids:

- 10.2.1 To analyze solids, chose a sample size that will ensure a representable sample. In general, weigh out 10 grams for soils and 1 gram for sludges. The sample should be broken up with a sterile transfer loop and then added to 100 mL of sterile *dilution water*. *The sample mixture should be allowed to soak for 10 minutes and shaken repeatedly to ensure a homogenous mixture.*

- 10.2.2 Depending on the type of sample, different serial dilutions may be considered. Dry soils are generally inoculated at 1 mL to 0.01 mL. Dried sludges are started at 1 mL and diluted to 0.001 mL.

11.0 Daily reading and recording of incubated samples.

- 11.1 Presumptive Phase: The tubes are incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. 24 ± 2 hours after the sample has been added into the tubes of sterile broth, the tubes are removed from the incubator and examined. A negative result will show no CO_2 formation inside the Durham tube. The negative broth tubes are re-incubated for another 24 ± 3 hours. After the total 48 ± 3 hours of incubation shows no CO_2 formation in the Durham tubes, the test is completed and the sample is considered negative for coliform bacteria. The broth tubes which do contain CO_2 bubbles are considered presumptively positive for coliform bacteria and proceed to the confirmed phase. Any sample producing turbid cultures (heavy growth) or acidic reaction (significant color change) is automatically transferred to confirming media.
- 11.2 Confirmed Phase: Each broth tube which shows CO_2 formation inside the Durham tube is transferred to a brilliant green bile tube. Insert a transfer loop into the positive tube, stir, and then place loop into the brilliant green bile tube. These tubes are incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for a total of 48 ± 3 hours. If gas formation is present in their Durham tubes when examined after 24 ± 2 or 48 ± 3 hours, the sample is considered positive for total coliform bacteria. The positive broth tubes are also transferred to E.C. media (wastewaters), or E.C. + MUG (drinking waters). The tubes of media also contain Durham tubes. After the sample has been transferred to the E.C. tubes, the tubes are incubated at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ for only 24 ± 2 hours in the fecal water bath. Tubes are placed in the water bath within 30 minutes of inoculation. If CO_2 formation is observed in the EC tube after the incubation period, the sample is considered positive for fecal coliform bacteria. EC tubes that are so turbid that the Durham tube is difficult to see are considered positive if there is foam on the top and the Durham tube sounds hollow. If CO_2 formation is observed in the EC + MUG tube and the tube fluoresces in UV light, the sample is considered positive for *E. coli*. Any tube that is positive for fecal coliform bacteria or *E. coli* must also be positive for total coliform bacteria.
- 11.3 The following table is a summary of expected media results.

12.0 Quality Control:

Note: See also ESB SOP B01 for general QC requirements

- 12.1 Positive and Negative controls:
- 12.1.1 To check for the possible occurrence of false positives due to fecal water bath malfunction during a fecal coliform test, one EC fermentation tube is inoculated with *Enterobacter aerogenes*. Another tube is inoculated with *Pseudomonas aeruginosa*. They are incubated for 24 ± 2 hours. If the fecal water bath has maintained a constant temperature of $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$, the *E. aerogenes* and *P. aeruginosa* will no longer be viable and will not produce gas.
- 12.1.2 As an additional fecal water bath QC check, another EC fermentation tube is inoculated with *Escherichia coli* and is incubated for 24 ± 2 hours. If the water bath temperature has been properly maintained, the *E. coli* will produce gas.

12.1.3 A fourth tube of EC media is incubated. It is a blank to ensure sterility.

12.2 Duplicates:

12.2.1 Sample duplicates are performed at a frequency of 5% or once per batch (day) whichever is more.

12.2.2 A precision criterion is generated from historical data as described in Standard Methods 9020:VII. Results ≥ 10 times the reporting limit are evaluated against the precision criterion. Attach the qualifier N-Brp to duplicates that do not meet acceptance criteria. If duplicates are extremely erratic, report the higher result with the qualifier N-Bru.

12.3 *Completed Phase*: The completed test is run on a quarterly basis.

12.3.1 A completed test is used to establish definitely the presence of coliform bacteria and to provide quality control data. A positive green bile tube containing CO₂ is streaked on an eosin methylene blue agar dish. The streaking method must ensure presence of some discrete colonies after a 24 ± 2 hour incubation period at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The colonies which grow on the E.M.B. agar dish fall in two categories: typical and atypical. The typical colonies are nucleated, with or without a metallic sheen. The atypical colonies are unnucleated, opaque, and mucoid. From the plate, pick one or more typical, well isolated colonies and transfer to a tube of lauryl sulfate broth. Incubate this tube at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours. If no gas production is observed, reincubate for an additional 24 ± 3 hours. Formation of gas within this lauryl sulfate broth tube within the 48 ± 3 hour incubation period demonstrates the presence of a member of the coliform group.

12.3.2 Completed test results are recorded.

12.4 Media Checks:

12.4.1 A blank, known negative and a known positive culture are analyzed with each set of samples. Tubes of Lauryl Sulfate Broth are inoculated each with *E. coli* and *P. aeruginosa*. These are incubated along with the Lauryl Sulfate Broth samples to ensure reproducibility of results. This is also done with green bile and incubated along with the green bile tubes to ensure reproducibility.

13.0 Calculations and Record Keeping

13.1 The 24 and 48 hour reading times are recorded under a "24hr" and "48hr" heading by the microbiologist. Documentation is placed on a data sheet containing time and date sample is planted, making the reading dates self evident. The MTF date and time read is documented in the test report and appears in the final report when appropriate.

13.2 **Most Probable Number (MPN)** The most probable number estimates the number of specific bacteria in water and wastewater by the use of probability tables (see attachments). The MPN is based on the number of tubes that have confirmed for or are present for bacteria (i.e. total coliform, fecal coliform, etc.). The MPN can also be determined by a combination of confirmed tubes when using multiple dilutions. When more than three dilutions are analyzed, use the highest dilution (most diluted sample aliquot) that gives confirmed results in all five test tubes along with the next two dilutions. Probability tables are found in Standard Methods, 22nd edition and are based on the amount of bacteria per 100 mL of sample. If the combination of positive tubes cannot be found on the MPN charts, the MPN can be calculated by the following:

$$\frac{\text{Number of Positive Tubes} \times 100}{\left(\frac{\text{mL of sample in negative tubes}}{\text{mL of sample in all tubes}} \right) \times \left(\frac{\text{mL of sample in all tubes}}{\text{mL of sample in negative tubes}} \right)}$$

This formula was found in Standard Methods, 22nd edition.

13.2 Dilution Factors for Aqueous samples

Liquids: 1mL = MPN X 10

0.1mL

0.01mL

0.1mL = MPN X 100

0.01mL

0.001mL

0.01mL = MPN X 1,000

0.001mL

0.0001mL

13.3 Calculation of Solids

Equation for figuring out MPN for solids:

$\frac{1 \text{ Gram}^*}{\text{Grams of solids used}} \times \text{Dilution Factor}^{} \times \text{MPN from chart}$**

*** final results always based on 1 gram**

**** 1mL, (X10), 0.1mL (X 100) See section 13.2.**

For example : SOLIDS, USING 10 GRAMS:

1mL = $\frac{1g}{10g} \times 10 \times \text{chart MPN} = \text{result}$
0.1mL
0.01mL

0.1mL = $\frac{1g}{10g} \times 100 \times \text{chart MPN} = \text{result}$
0.01mL
0.001mL

14.0 Method Performance

14.1 Refer to in-house quality control performance records.

15.0 Corrective Action For Out of Control Or Unacceptable Data:

- 15.1 If any of the above QC samples do not meet acceptance criteria, the analyst must take measures to correct the problem.
 - 15.1.1 The analyst examines the results of other controls performed that day.
 - 15.1.2 The analyst examines sample results either for historical trends or for none detects.
 - 15.1.3 If the above investigation indicates that the batch is still valid and the out of control QC appears to be an anomaly, results are reported.
 - 15.1.4 If it is unclear whether sample results can be verified, any affected samples must either be resampled or results must be reported with a note qualifying the data.
 - 15.1.5 Out of control QC is noted on the QC data page along with the corrective action taken. A QC follow up form is filled out in the computer and added behind the QC data page.

16.0 Pollution Prevention and Waste Management

- 16.1 All positive samples and QC are autoclaved prior to disposal.
- 16.2 See also SOP S07 – Pollution Prevention

References

Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WPCF, 22nd edition. 9221 B,C,E.

Official Methods of Analysis of AOAC International, 17th Edition.

BAM-FDA 8th Edition.

Note: All *italicized items* except bacteria names are an indication of a variation from the method.

Approved by Stacey A. Fry Date 10/13/2013

TABLE 9221.III. MPN INDEX AND 95% CONFIDENCE LIMITS FOR
VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS
WHEN TEN 10-mL PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 10 of 10 mL Each	MPN Index/ 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	< 1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	>23.0	13.5	Infinite

TABLE 9221.IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 mL, 1.0 mL, 0.1 mL)

Combination of Positives	MPN Index/ 100 mL	95% Confidence Limits		Combination of Positives	MPN Index/ 100 mL	95% Confidence Limits	
		Lower	Upper			Lower	Upper
0-0-0	< 2	—	—	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	10	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1	33	15	77
1-0-0	2	1.0	11	4-4-0	34	16	80
1-0-1	4	1.0	15	5-0-0	23	9.0	86
1-1-0	4	1.0	15	5-0-1	30	10	110
1-1-1	6	2.0	18	5-0-2	40	20	140
1-2-0	6	2.0	18	5-1-0	30	10	120
2-0-0	4	1.0	17	5-1-1	50	20	150
2-0-1	7	2.0	20	5-1-2	60	30	180
2-1-0	7	2.0	21	5-2-0	50	20	170
2-1-1	9	3.0	24	5-2-1	70	30	210
2-2-0	9	3.0	25	5-2-2	90	40	250
2-3-0	12	5.0	29	5-3-0	80	30	250
3-0-0	8	3.0	24	5-3-1	110	40	300
3-0-1	11	4.0	29	5-3-2	140	60	360
3-1-0	11	4.0	29	5-3-3	170	80	410
3-1-1	14	6.0	35	5-4-0	130	50	390
3-2-0	14	6.0	35	5-4-1	170	70	480
3-2-1	17	7.0	40	5-4-2	220	100	580
4-0-0	13	5.0	38	5-4-3	280	120	690
4-0-1	17	7.0	45	5-4-4	350	160	820
4-1-0	17	7.0	46	5-5-0	240	100	940
4-1-1	21	9.0	55	5-5-1	300	100	1300
4-1-2	26	12	63	5-5-2	500	200	2000
				5-5-3	900	300	2900
				5-5-4	1600	600	5300
				5-5-5	≥1600	—	—

Sample Acceptability
Standard Operating Procedure
Babcock Laboratories, Inc.
Effective Date 6/27/2014

- 1.0 All information accompanying a sample must be recorded in permanent ink.
- 2.0 Sample Documentation
 - 2.1 Bottle label
 - 2.1.1 Sample identification or Location
 - 2.1.2 Date and time of collection
 - 2.1.3 Sampler's name
 - 2.1.4 Preservation type
 - 2.1.5 Sample type
 - 2.2 Accompanying paperwork
 - 2.2.1 Items listed above in section 2.1
 - 2.2.2 Any special remarks concerning the sample
- 3.0 At Log-in the laboratory affixes a durable, water-resistant label on each sample bottle, which bears a unique identification (lab number, preservation note, and sample storage/cart location). Client sample label information is verified against laboratory label information to ensure labels match.
- 4.0 Sample Conditions:
 - 4.1 Each member of our field department has a detailed knowledge of proper sampling techniques and sample handling procedures and understands the criteria for sample acceptability. Samples are collected in approved containers.
 - 4.2 Clients are informed of proper sampling, transportation, preservation, and holding time information. Containers and preservatives are provided upon request.
 - 4.3 Inorganic and Organic Analyses:
 - 4.3.1 See ESB SOP Q14 for Inorganic and Organic Container, Volume, Preservation, and Holding Time information.
 - 4.3.2 See individual methods for additional criteria.

4.4 Bacterial Analysis:

- 4.4.1 Sterilized containers and lids are utilized for all bacterial analyses. A capacity of at least 100 ml is required but containers are not overfilled in order to allow room to shake and homogenize samples. Sterilized containers supplied by the laboratory are used whenever possible. If the client provides the containers, boiling of the container and lid for 20 minutes is requested. Sample containers are placed inside Ziplock bags at the time of sampling or pick up so that they are not contaminated by moisture or melting ice. Containers are to remain in Ziplock bags until relinquished to sample receiving personnel. See SOP F02a for guidelines in Bacteriological sampling.
- 4.4.2 The sample is kept on ice (blue ice) or refrigerated until it arrives at the laboratory.
- 4.4.3 The time from collection until the sample is incubated does not exceed 30 hours for potable waters and 8 hrs for wastewaters, nonpotable groundwaters and surface waters.

5.0 Sample Acceptance Criteria:

If the sample does not meet the acceptance policy, a notation as to the variation and client communication is made on the Chain of Custody or Sample Receipt Form.

Note: If a regular client has instructed the laboratory in the past to proceed with analysis on specific samples, despite sample acceptance deviations, the laboratory may apply this permission to future samples posing the same concerns. A Sample Acceptability Waiver Form is kept on file to document their permission to proceed under specific conditions. Samples still bear a qualifier explaining the acceptability problem. See section 9.0 for a copy of the Waiver form.

Note: For some samples, (e.g. storm water samples) it may be impossible to resample or acquire more sample volume. Since this is known by the laboratory in advance, the laboratory is likely to proceed with analysis, without client notification, regardless of acceptance criteria concerns.

A Sample Acceptability checklist will be posted in the login area as a reminder of the following criteria:

5.1 Proper containers:

- 5.1.1 The sample must not cause the container to corrode and the container must not contaminate the sample. It must be of sufficient volume to hold enough sample for the required analyses--if not, multiple containers may be used.
- 5.1.2 For most uses, containers may be made of LPE plastic. Soda glass or borosilicate with Teflon is used for organic sampling. Plastic Ziplock bags or Mason jars are acceptable for many types of solid samples. For source gas emissions, tedlar bags, canisters, and absorbent traps are commonly used.
- 5.1.3 The containers must not be leaking or damaged.
- 5.1.4 Sample containers for bacteriological analysis should be in Ziplock bags.
- 5.1.5 The client must verbally authorize the lab to proceed with analysis if samples are received in improper containers. The authorization must be noted on the Sample Receipt Form or Chain of Custody.
- 5.1.6 A note is placed on the laboratory report using a sample qualifier or case narrative stating that the sample was received in an inappropriate container.

5.2 Sample preservation:

- 5.2.1 If the sample is received unpreserved the Splitter will subsample and preserve as needed. Documentation is noted on the Laboratory Work Order.

- 5.2.2 If the sample is received with improper preservation, the client must verbally authorize the lab to proceed with analysis. The authorization must be noted on the Sample Receipt Form or Chain of Custody.
- 5.2.3 A note is placed on the laboratory report using a sample qualifier or case narrative stating that the sample was improperly preserved.
- 5.3 Sample Temperature:
 - 5.3.1 Samples requiring thermal preservation from sampling until analysis as stipulated by the method are as follows:
 - 5.3.1.1 Nonmetal aqueous Chemistry analyses - above freezing to 6°C.
 - 5.3.1.2 Wastewater Bacteriological analyses - above freezing to <10°C.
 - 5.3.2 An arrival temperature is taken of a representative sample from each client delivery and recorded on the Chain of Custody or Sample Receipt Form or if available, a cooler blank (CB) temperature is taken. The temperature is recorded as “Not Available” if it is not taken immediately upon removal from the ice chest. See guidelines in A03 Sample Receiving and Login SOP. The arrival temperature of a representative sample or cooler blank is taken and recorded by pointing infrared gun at surface of the sample bottle.
 - 5.3.2.1 To operate infrared gun:
 - 5.3.2.1.1 Press and continue to hold down the trigger to take temperature readings. The unit will take temperature readings while the trigger is pressed and held (SCAN appearing on the display indicates measurements are being taken).
 - 5.3.2.1.2 Once trigger is released the last temperature will be displayed for approximately 15 seconds and the display will turn off automatically.
 - 5.3.2.1.3 HOLD will appear on the display when the trigger has been released to indicate unit is no longer taking the temperature measurement.

5.3.2.1.4 Laser targeting is always active when the trigger is being pressed. (Caution: Eye damage may result from direct exposure to laser light.) The target must completely fill the spot diameter seen by the infrared sensor; otherwise readings will be influenced by the surface surrounding the target.

5.3.2.2 Avoid sample label when aiming infrared gun.

5.3.2.3 Record temperature reading to the nearest whole number.

5.3.2.4 The infrared gun is calibrated annually against a NIST certified thermometer. See SOP Q04 Section 5.0 for calibration procedures.

5.3.3 For samples that arrive on ice, hand delivered on the same day that they are collected; it is assumed that the cooling process has begun regardless of the arrival temperature.

5.3.4 The client is advised that the sample temperature may affect test results if a nonmetal aqueous Chem sample arrives at: $>6^{\circ}\text{C}$ or a wastewater Bacti sample arrives: $\geq 10^{\circ}\text{C}$ and is one of the following:

5.3.4.1 Without ice or

5.3.4.2 Sampled on a different day (i.e. delivered by mail)

5.3.5 If a sample arrives as explained in section 5.3.4, the client must verbally authorize the lab to proceed with analysis. The authorization must be noted on the Sample Receipt Form or Chain of Custody.

5.4 Sample Holding Time:

5.4.1 A note is placed on the laboratory report using a sample qualifier that states that the sample was received past the regulatory holding time for a particular analysis.

5.5 Adequate Sample Volume:

- 5.5.1 The client may be asked to verbally authorize the lab to proceed with analysis if adequate sample volume is not provided. In many cases analysis is performed using less sample volume without authorization. However, if the sample size provided requires a drastic reduction in analysis aliquot, then the client will be called for authorization, with the possible exception of storm water samples where sample volume cannot be controlled.
- 5.5.2 Sample results are reported with elevated reporting limits to reflect diminished sample volume.

5.6 Litigation Samples:

- 5.6.1 The client must verbally authorize the lab to proceed with analysis if samples are not properly sealed. The authorization must be noted on the Sample Receipt Form or Chain of Custody.
- 5.6.2 A note is placed on the laboratory report in the case narrative stating that evidence tape was not intact upon receipt to the laboratory.

5.7 Litigation Samples/ Chain of Custody Forms:

- 5.7.1 A Chain of Custody form is required whenever the potential exists that the sample may be used for litigation. This form contains all information included on the sample label as well as "Relinquished by" and "Received by" blocks for the name of the person who submits or releases the sample and the name of the person who receives the sample along with the date and time that the custody of the sample changes hands.
- 5.7.2 If samples are not accompanied by a Chain of Custody form, the client is given the opportunity to complete one. If the client does not wish to do so, analysis may proceed using a Sample Receipt Form.

- 5.8 The Chain-of-Custody information must agree with the sample labels that are received.
 - 5.8.1 The client must verbally authorize the lab to correct the discrepancy if the Chain-of-Custody or sample label is incorrect. The authorization must be noted on the Chain-of-Custody.
 - 5.8.2 If complete information is not provided, every attempt is made to contact the client. Communication is noted on the Chain of Custody or Sample Receipt Form.
- 6.0 Sample Rejection Policy:
 - 6.1 At the laboratory's discretion, a sample may be rejected if it cannot be analyzed under the conditions at which it was submitted, due to the nature of the analysis requested. For example:
 - 6.1.1 Total Coliform by SM 9223, if less than 100mL of sample is submitted.
 - 6.1.2 Nitrite analysis on a nitric preserved sample.
 - 6.1.3 Sulfate analysis on a sulfuric preserved or sulfate salt preserved sample.
- 7.0 Log-in personnel will note any further abnormalities observed during sample receipt on the Chain of Custody.
- 8.0 Laboratory personnel are informed via notes in the sample comment section if samples are not preserved properly, incorrect containers are used, inappropriate sample size is provided, holding times have been exceeded, or any other problems occur. These comments alert the analyst in case special treatment is required for analysis due to the incorrect sampling conditions.
- 9.0 All requested analyses must be understood and acceptable. If further clarification is needed the client must be contacted.

10.0 If the client is contacted for any of the above reasons the following is noted on either the Sample Receipt Form or Chain of Custody:

- 10.1 Person contacted
- 10.2 Date
- 10.3 Problem
- 10.4 Resolution
- 10.5 Initials

11.0 Additional Requirements for UCMR3 Program

11.1 Samples received from the EPA are examined upon arrival to ensure that the Kit number on the shipping box and the tracking form match the number on the sample bottles. Sample receiving personnel refer to the **Kit Nomenclature sheet** to ensure proper matching of kit numbers.

11.2 Samples are examined to ensure that proper shipping conditions were met:

11.2.1 Samples must be on ice or with gel packs

11.2.2 Samples must not be frozen

11.2.3 Samples must be protected from light

11.3 The temperature is taken of a representative bottle immediately upon arrival. Samples will be invalidated if they are not received or stored at the proper temperature. See section 5.3 for temperature procedure:

11.3.1 Samples must be $\leq 10^{\circ}\text{C}$ unless sampled that day and placed on ice

11.3.2 Samples must be $\leq 6^{\circ}\text{C}$ if sampled more than 48 hours before receipt at the laboratory.

11.3.3 Samples are to be refrigerated at 6°C or less after arrival at the laboratory

11.4 Samples are examined to ensure proper containers and preservations were used. Refer to the **Sample Containers, Preservation and Holding Times for UCMR3** table in SOP Q14 for details. If improper containers and/or preservatives are used, or if inadequate sample volume is provided, samples will be invalidated and the water system will be notified so that a replacement sample can be collected.

11.4.1 Sample vials for EPA Method 524.3 are checked to ensure that headspace is $\leq \frac{1}{4}$ inch in diameter.

11.5 Samples are checked for appropriate use of Field Blanks, where applicable. Clients are advised to return empty reagent water bottles used for Field Blanks and reagent vials to the laboratory at the time of sample submittal. For samples requesting methods 537 and 539, sample receiving personnel check to ensure that the Field Blank containers are full and the reagent water bottles are empty. For method 522, reagent vials are checked to ensure they are empty. If the empty reagent water bottles and/or reagent vials are not submitted to the laboratory, a note is documented on the Chain of Custody.

11.6 The laboratory verifies the pH and Chlorine of each sample submitted to the laboratory on the day of receipt. Refer to SOP A06 for details on the screening procedure.

11.6.1 If the pH is not within range or chlorine is present ($\geq 0.1\text{mg/L}$), the samples must not be analyzed. The laboratory will request a resample. If resampling cannot be performed, the PWS must indicate in the report to the EPA that the samples were invalidated due to a sampling error and no data will be reported.

11.6.1.1 For Method 200.8, if $\text{pH} > 2$ the sample can be preserved at the laboratory but must be held for at least 16 hours prior to analysis.

11.6.1.2 For Method 522, additional sodium bisulfate may be added to bring $\text{pH} < 4$ upon receipt to the laboratory, if sample was collected within 48 hours of sample delivery.

11.7 The information listed on sample bottle labels is compared to information listed on the accompanying Chain of Custody to check for accuracy. The following information must accompany each sample submitted to the laboratory:

11.7.1 Sample Point Identification Code

11.7.2 Sampling date and time

11.7.3 Sample Event Code (SE#)

11.7.4 Public Water System Identification Code

11.7.5 PWS Facility Identification Code

Approved by Stacey A. Fry Date 6/18/2014

12.0 Sample Acceptability Waiver Form



E.S.BABCOCK & Sons, Inc.
Environmental Laboratories est. 1906

Sample Acceptability Waiver Form
E. S. Babcock & Sons, Inc.

Client: _____

This waiver applies to the following:

Sample I.D., Project, or All future samples (circle one)

Sample Acceptability Problem:

Improper containers _____
Improper preservative _____
Improper temperature _____
Received past holding time _____
Other: _____

I authorize E.S. Babcock & Sons to continue present and future analyses for samples described above, even if they do not meet the sample acceptance requirements noted above each time they are submitted.

Signature: _____

Print Name: _____

Date: _____

mailing
P.O. Box 432
Riverside, CA 92502-0432

location
6100 Quail Valley Court
Riverside, CA 92507-0704

P 951 653 3351
F 951 653 1662
www.babcocklabs.com

NELAP no. 02101CA
CA ELAP no. 1156
EPA no. CA00102

13.0 Kit Nomenclature Sheet

Kit Nomenclature

For kits where we expect an LFSM/LFSMD analysis, your lab will receive a kit with a "Q" suffix, and a kit with a "D" suffix. They will have the same six numeric ID (i.e., you would get a 199999Q and a 199999D). OR, for referee kits, you would get a kit with an "F" and a kit with a "D" (i.e., 199998F and 199998D).

The FS and LFSM kits should come from the "Q" (or "F") kit.
The LFSMD kits should come from the "D" kit.

If you do not have enough volume, or the LFSM has a QC failure, please inform Karen or Susie and we will discuss this with the EPA.

Additional Notes

Kits That Start With The Following Numbers

- 1 - AM (EP)
- 3 - AM (MR)
- 5 - SS (EP)
- 8 - Pilot or Blind Studies
- 9 - PST - If you see a kit with a 9, contact EPA immediately. Those kits are for virus sampling and you received this kit by mistake.

Kits That End With The Following Letters

- P - Primary (FS)
- Q - QC (FS) (LFSM)
- D - QC - (LFSMD)
- R - Resample (FS)
- F – Referee (Sample points analyzed in duplicate - there would be a "P" kit and a matching "F" kit that are shipped to two different labs)
- N - PST - Indicators. If you see a kit that ends with an N, contact EPA immediately. Those kits are for virus sampling and you received this kit by mistake.

RESIDUE, TOTAL SUSPENDED
Babcock Laboratories Inc.
STANDARD OPERATING PROCEDURE
(SM 2540 D)

Date Effective: __6/27/14__

1.0 Scope and Application:

- 1.1 This method is applicable to all aqueous samples.

2.0 Working Range:

- 2.1 The working range is 5mg/L (the reporting limit) to 2000mg/L.

3.0 Method Summary:

- 3.1 A measured volume of sample is filtered through a pre-tared filter. The residue that remains on this filter after drying in a 105°C oven is considered the suspended solid portion of the sample.

4.0 Sample Collection, Preservation and Holding Time:

- 4.1 The sample must be unpreserved. It must be stored at 4°C until analysis. Analysis must take place within 7 days of sampling per CFR part 136, Table II.

5.0 Interferences:

- 5.1 Non-representative particulates such as leaves, rocks, and sticks may be excluded.
- 5.2 To avoid water entrapment, limit the sample size to that yielding no more than 200 mg residue on the filter. (This would be a final result of 2000 mg/L since we are analyzing 100 mLs of sample.)
- 5.3 For samples high in dissolved solids thoroughly wash the filter with DI water after the sample has passed through the filter.
- 5.4 Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter.

6.0 Apparatus, Standards and Reagents

- 6.1 Side-arm flask of sufficient capacity for sample size selected.
- 6.2 Filtration apparatus: Membrane filter funnel with a Whatman grade 934-AH glass fiber filter disk (47mm) with a suitable diameter for the funnel.
 - 6.2.1 Each new lot of filters is tested before use. Prepare three filters by rinsing three times with DI water and heating at 105°C for an hour. Take out, let cool, and record tare weight. Place again in the 105°C oven for an hour. Take out, let cool, and record final weight. The difference between tare and final weight should be within MB criteria. See section 9.2.
- 6.3 Drying oven, for operation at 105°C \pm 2°C.
- 6.4 Vacuum aspirator. Monitor oil level and fill when necessary.
- 6.5 Desiccator – Check on the desiccant to make sure that it is always blue. Replace desiccant when it turns purple.
- 6.6 Balance #58 with a sensitivity of 0.1 mg. Calibrated with 0.1 g, 5g, and 100 g class "S" weights on a daily basis. Calibration must be within 5 mg (for 5g & 100g) and 0.5 mg (for 0.1g). If values are not within these limits, recalibrate the balance.
- 6.7 *Aluminum pans* to hold glass fiber filters.
- 6.8 Standard laboratory glassware: volumetric flasks, graduated cylinders, pipettes.

Note: All glassware is cleaned immediately prior to and after use by thorough rinsing with three portions of DI water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 acid rinse, acetone or appropriate solvent rinse. Glassware is always finished with a final DI rinse.

- 6.9 Stock Standards:
 - 6.9.1 Cellulose – stored at room temperature for up to 10 years from date received.
- 6.10 Lab Control: 500 mg of cellulose is weighed into a liter of DI water. This solution is kept at room temperature for up to 6 months. Use a 100 mL aliquot.
- 6.11 Reagent water: DI water.

7.0 Procedure

- 7.1 Each box of glass fiber filters are prepared by rinsing three times with DI water and then dried to a constant weight. Rinsed filters are placed on a tray and heated at 105°C for a minimum of 1 hour. The tray is then removed and cooled to room temperature and then placed in a desiccator. The tray is then weighed and weight recorded. The tray is put back in the oven for a minimum of 15 minutes then removed, cooled and weighed again. This process is repeated until a constant weight is achieved. Lot #, constant weight and date of preparation are noted on bench sheet.
- 7.2 Use forceps when handling filters. Place filter on balance and record tare weight. Place the filter onto the filtering apparatus. Wet filter with a small amount of DI to seat it.
- 7.3 Mix sample well by *shaking* sample bottle. Measure an appropriate volume of sample in a graduated cylinder, normally 100 mL. Use a 200 mL sample volume for method blank and samples expected to contain very minute amounts of suspended material. Upon client request a larger volume of sample up to 1000 mL will be used. Filter through apparatus collecting suspended residue on filter. Rinse cylinder and filter 2 to 3 times with a small amount of DI water. Apply suction for three minutes or *until no visible free liquid is present*.
 - 7.3.1 For samples with a lot of suspended matter, a smaller volume of sample (10 – 50 mL) may be used. **Avoid using less than 5 mL.** If less than 10 mLs must be used, an auto pipette can be used to obtain a representative sample by carefully cutting the tip. Verify the accuracy of the altered tip by using the scale. Make sure the tip is rinsed well.
- 7.4 Place filters in 105°C for a minimum of one hour.
- 7.5 Remove filters from oven. Cool filters in an area free of moisture or contamination for 10-15 min. Weigh filters or place in a desiccator until ready to weigh.
- 7.6 Weigh on a balance. Record the weight. Place filters back in the oven for a minimum of 15 minutes, remove, cool and then weigh again. Record the weight.
 - 7.6.1 This process is repeated until the weights are constant. Constant weight is defined as; “weight change less than 4% of the previous weight or a change less than 0.5 mg, whichever is less” (basically the last digit in the weight cannot vary by more than 5).
- 7.7 Use the last final weight taken for each sample to calculate result.

8.0 Calculation and Reporting:

$$\frac{(A-B) \times 1,000,000}{\text{\# mL of sample used}}$$

Where A = Weight in grams of filter with residue, and

B = Tare weight in grams of filter.

8.1 Alternatively, you may subtract the actual numbers in the weight readings (without any decimal points) and multiply the difference by the factor of 100/(mL of sample used).

8.2 Results are entered into LIMS by taking the

Difference (from data page) X Dilution Factor (based on a 200mL default volume)

2

8.3 The reporting limit for this procedure (based on a 200 mL aliquot) is 5 mg/L. Current MDL info can be found in Element.

8.4 Report all results to three significant figures.

8.5 The difference is entered as the initial raw result.

8.6 The dilution factor, based on a 200 mL sample aliquot, is entered into the Dil column in LIMS.

8.7 The analyzed time is documented as the time the last sample in the batch was filtered. This is equal to the time filters are placed in the 105°C oven.

8.8 Set the prepared time to equal the analyzed time in Element

9.0 Quality Control:

Note: See also ESB SOP Q01 for general QC requirements

9.1 Duplicates are run with every batch or one every ten samples per matrix type, whichever is more frequent. The Relative Percent Difference is calculated. If the RPD does not fall within the acceptance limit maximum of 25%, the sample is re-analyzed. If the RPD still does not fall within the acceptance range, a note is placed on the client's results.

9.2 A method blank is analyzed with every batch of samples, at a minimum of once for every 20 samples and reported per matrix type. Results must be less than 5 mg/L and greater than -5 mg/L for batch acceptance. Sample results at or above the reporting limit must be accompanied by a qualifier if the method

blank exceeds a concentration greater than 1/10 of the measured raw concentration of the sample. Blank results below the RL are considered to be ND and will not require a note.

- 9.3 A lab control is analyzed with every batch at a minimum of once for every 20 samples and reported per matrix type. Results must be between 90-110% recovery for batch acceptance. If the result falls outside the control limits, the analysis is considered to be out of control and all other results in the analytical run are questionable. Troubleshooting is performed to attempt to determine the cause. If the cause is not determined, or the cause is something that could affect the other samples in the batch, the analyses will be re-run.
- 9.4 An MDL study is completed whenever major equipment or procedural changes are made. Standards should be spiked at 2.5-5 times the estimated MDL. A minimum of six to seven replicates is analyzed. See QA Manual for calculation. Results must be below the reporting limit.
- 9.5 LOD Verification: On a yearly basis, a QC sample is spiked at a level of 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.
- 9.6 Initial Demonstration of Capability: Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four consecutive Lab Control Samples. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 20.
- 9.7 Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

10.0 Corrective Action For Out of Control Or Unacceptable Data:

See SOP Q06 – Corrective Action

11.0 Pollution Prevention and Waste Management:

SOP S07 – Pollution Prevention

12.0 Definitions: See SOP Q15 – SOP Definitions

13.0 Safety

13.1 General laboratory safety procedures are sufficient for this analysis. Recommended safety equipment includes gloves and safety glasses.

14.0 Method Performance:

Refer to MDL studies, Initial Demonstration of Capability Studies, and laboratory control charts maintained in the QC Office.

15.0 References

Standard Methods For the Examination of Water and Wastewater 22nd Edition APHA/AWWA/WEF 2540D.

HR SERIES Instruction manual 1596-5A-IE-99.03.26

Note: All *italicized items* are an indication of a variation from the method.

Approved by: Julia Sudds Date: 6/19/14

T-303-NH3N

Nitrogen, Ammonia (Colorimetric, Automated Phenoate)

Standard Operating Procedure

SM 4500-NH₃ G

Effective Date: 12/18/2015

Revision#: 1.0

Approved by: _____

(signature/date)

Meets Quality System Requirements: _____

(signature/date)



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The Standard of Excellence for Over 100 Years

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1.0 SCOPE AND APPLICATION

- 1.1 This method covers the determination of ammonia in drinking, surface, and saline waters, domestic and industrial wastes in the range of 0.1 to 2.0 mg/L NH₃ as N. This range is for photometric measurements made at 660 nm in a 10 mm tubular cell. Higher concentrations can be determined by sample dilution. This method may also be used for solid samples if both client and regulator agree to this variation.
- 1.2 Environmental Relevance: Ammonia is present naturally in surface and wastewaters. Its concentration is generally low in ground waters because it adsorbs to soil particles and clays and is not leached readily from soils. It is produced largely by deamination of organic nitrogen containing compounds and by hydrolysis of urea. At some treatment plants ammonia is added to react with chlorine to form combined chlorine residual. In the chlorination of wastewater effluents containing ammonia, virtually no free residual chlorine is obtained until ammonia has been oxidized. The chlorine reacts with ammonia to form mono and dichloramines.
- 1.3 Ammonia is the most reduced form of nitrogen and is found in water where dissolved oxygen is lacking. When dissolved oxygen is readily available, bacteria quickly oxidize ammonia to nitrate through nitrification. Other types of bacteria produce ammonia as they decompose dead plant and animal matter. Depending on temperature and pH, high levels of ammonia can be toxic to aquatic life. High ammonia concentrations can stimulate excessive aquatic production and indicate pollution. Important sources of ammonia to lakes and streams can include: fertilizers, human and animal wastes, and by-products from industrial manufacturing processes.

2.0 SUMMARY OF METHOD

- 2.1 Samples are distilled (if under NPDES program) or filtered and analyzed on an automated colorimetric system. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside

3.0 DEFINITIONS- See Definitions Appendix in Quality Manual

4.0 SAFETY

- 4.1 Identified Hazards
 - 4.1.1 Normal, accepted laboratory safety practices should be followed during reagent preparation and instrument operation. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.
- 4.2 SDS Review: Safety Data Sheets are available for items listed under section 8.0 if applicable. The following chemicals have the potential to be highly toxic or hazardous, consult SDS.
 - 4.2.1 Sulfuric Acid
 - 4.2.2 Phenol
 - 4.2.3 Sodium nitroprusside

4.3 Safety Information about Enviro Midi-Dist Distillation System

- 4.3.1 Do not operate the Enviro Midi-Dist in the vicinity of combustible material.
 - 4.3.2 During operation the surfaces around the heater assembly will get HOT- Do not touch the outer surface.
 - 4.3.3 Do not move the Enviro Midi-Dist system while hot.
 - 4.3.4 Do not attempt to operate the Enviro Midi-Dist system over 190 °C.
 - 4.3.5 Allow the Enviro Midi-Dist to cool for 20minutes before removing glassware. Separation of the "hot" glassware components could result in bumping, boil over, and/or spraying of hot corrosive/caustic materials.
 - 4.3.6 For more information see the Instruction Manual for the Enviro Midi-Dist Distillation System.
- 4.4 Required PPE: Employees are required to wear a lab coat, safety glasses, and appropriate gloves when working in the lab or handling samples, standards, acids or bases.
- 4.5 Required training: Review of safety data sheets, PPE, and fume hood training
- 4.6 Safety precautions noted throughout procedure are noted as **SAFETY NOTES**.

5.0 INTERFERENCES

- 5.1 Calcium and magnesium ions, may be present in concentrations sufficient to cause precipitation problems during analysis. A 7.5% EDTA solution is used to prevent the precipitation of calcium and magnesium ions from river water and industrial waste. For sea water, a sodium potassium tartrate solution may be used. (See SM 4500-NH₃ H, 3e. for recipe.)
- 5.2 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere. If necessary, sample is diluted.
- 5.3 Urea and cyanates will hydrolyze on distillation at pH of 9.5.
- 5.4 Dechlorination is not necessary since the colorimetric analysis will detect chloramines formation.
- 5.5 Marked variation in acidity and alkalinity are eliminated by sample preservation with H₂SO₄. The pH is then checked to ensure that it is <2. Due to the reducing nature of this environment, residual chlorine is not expected to be a problem. *The sample is neutralized prior to analysis by the addition of the first reagent which is a NaOH buffer.*
 - 5.5.1 For highly acidic samples the pH of the dilution water should be raised to approximately 11pH before adding the sample to avoid over diluting.
- 5.6 Distillation is required for all samples under the NPDES permit program. All other samples are filtered and may be distilled if the sample presents special matrix problems.

6.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

6.1 RESERVED

7.0 EQUIPMENT AND SUPPLIES

- 7.1 Test tube rack from SEAL.
- 7.2 13 x 100 mm disposable culture tubes.
- 7.3 SEAL Analyzer
- 7.4 Whatman 2 and Whatman 4 (11.0cm) filter paper or Gelman 0.45 micron disk filters.
- 7.5 100 mL beakers.
- 7.6 1 mL, 5 mL, 10 mL autopipettes.
- 7.7 25 mL, 50 mL, and 100 mL graduated cylinders.
- 7.8 Distillation apparatus:– Enviro Midi-Distiller Glastron, Inc.– Distiller Environmental Express.
- 7.9 VWR boiling chips 26397-409
- 7.10 Antifoaming Agent JT Baker B531-05

Note: Rinse all glassware prior to use with three portions of D.I. water. Immediately after use thoroughly rinse with three portions of D.I. water. If glassware still appears dirty, further steps are taken, by use of one of the following: Alconox and hot water, 1:1 HCl, acetone or appropriate solvent rinse. Glassware is always finished with a final D.I. rinse.

8.0 REAGENTS AND STANDARDS

- 8.1 Reagent Water: D.I. water
- 8.2 Sulfuric Acid solution: concentrated. Store at room temperature for up to 10 years.
- 8.3 Diluent or preserved water: Add 1 mL of Sulfuric acid to 1 L of nanopure water. This solution is stored for up to 6 months at room temperature.
- 8.4 Sodium phenate: To a 100 mL beaker, add 2 g of sodium hydroxide and about 10 mL DI. Swirl to dissolve and cool to room temperature. In the fume hood, add 5 g of crystalline phenol and swirl to dissolve. Transfer to a 50 mL graduated cylinder and fill to the mark with DI water. Store in the refrigerator for three weeks or until the reagent becomes dark brown.
- 8.5 Sodium hypochlorite solution: bleach solution containing 6% NaOCl (such as "Clorox"). Store in refrigerator for up to 4 months.

- 8.6 Modified EDTA Buffer: Disodium ethylenediamine-tetraacetate (EDTA) (7.5%): Dissolve 18.75 g of EDTA (disodium salt) and 4.55 g of NaOH in 250 mL of nanopure water. This solution is stored for up to 6 months at room temperature.
- 8.7 Sodium nitroferriocyanide (0.2%): Dissolve 0.5 g of sodium nitroferriocyanide in 250 mL of D.I. water. This solution is stored for up to 1 month at room temperature in a dark bottle. Discard if the reagent becomes bluish in color.
- 8.8 1 N NaOH: Dissolve 40 g of NaOH into 1 L of D.I. water. This solution is stored for up to 6 months at room temperature.
- 8.9 0.04N Sulfuric Acid: Add 1.12 mL of concentrated sulfuric acid into 1 L of D.I. water. This solution is stored for up to 6 months at room temperature.
- 8.10 Hellma Hellmanex II: This solution is stored at room temperature for up to 10 years.
- 8.10.1 Add 2 mL of Hellmanex solution into 100 mL of D.I. water. This solution is kept at room temperature for up to 3 months.
- 8.11 Borate Buffer:
- 8.11.1 0.1 N NaOH: Dissolve 4 g of NaOH into 1 L of D.I. water.
- 8.11.2 Mix 0.95 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ into 100 mL of Nanopure water.
- 8.11.3 Add 8.8 mL of 0.1N NaOH and 50 mL of borate solution into a 100 mL flask and bring up to volume with D.I. water.
- 8.11.4 Solutions above are stored for up to 6 months at room temperature.

Note: The above reagent recipes are specified by the instrument manufacturer (SEAL Analytical AQ2 Method No: EPA 103-A Rev 4 © 6/01/05) and by Standard Methods 22nd Edition 4500-NH₃.

9.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 9.1 Preservation by addition of conc. H_2SO_4 to a pH < 2 and refrigeration at 4°C.
- 9.2 Samples must be analyzed within 28 days.

10.0 QUALITY CONTROL

10.1 Lab Control Sample (LCS)

- 10.1.1 Stock Solution: 1000 mg/L NH_4 Standard - stored at room temperature using manufacturer specified holding times. Once a stock standard is opened, the expiration date is one year as long as this date does not exceed the original manufacturer's date and percent recovery is acceptable.
- 10.1.2 LCS
- 10.1.2.1 Dilute 50 μL of stock standard (10.1.1) to 50 mL of diluent (8.3). The concentration is 1 mg/L NH_4 or 0.78 mg/L $\text{NH}_4\text{-N}$. (See below) This solution is stored at 4°C for up to 2 weeks.

- 10.1.2.2 An LCS is analyzed for every 20 samples per matrix type or one per analysis batch whichever is greater. LCSs are distilled unless the sample batch contains only undistilled samples.
- 10.1.2.3 If the LCS analysis does not fall within the acceptance range of 90-110%, the analysis is stopped until the cause is determined and the LCS is within the acceptance range.

$$1 \text{ mg/L NH}_4 \times \frac{14 \text{ g/mole N}}{18 \text{ g/mole NH}_4} = 0.78 \text{ mg/L N}$$

10.2 Matrix Spike (MS) / Matrix Spike Duplicate (MSD)

10.2.1 Spike Solution

- 10.2.1.1 Spike 50 uL of stock standard (10.1.1) into 50 mL of sample. The spike concentration is 1 mg/L NH₄ or 0.78 mg/L NH₄-N.
- 10.2.2 A MS/MSD is analyzed for every twenty samples per matrix type or one per analysis batch whichever is greater. Spikes are distilled unless the sample batch contains only undistilled samples.
- 10.2.3 Acceptability: Acceptance ranges are 80-120% recovery with a maximum RPD of 20%.

10.3 Method Blank

- 10.3.1 Use diluent from Section 8.3.
- 10.3.2 A MB is analyzed for every twenty samples per matrix type or one per analysis batch whichever is greater and reported per matrix type. Blanks are distilled unless the sample batch contains only undistilled samples.
- 10.3.3 Acceptability: MB must read below but not more negative than the RL of 0.1 mg/L for batch acceptance. Samples results reported must be accompanied with a note if the method blank exceeds a concentration greater than 1/10 of the measured raw concentration of the sample however, blank results below the RL are considered to be ND and will not require a note.

Note: The blank value noted in the NBLK qualifier is the raw blank result. If the sample being qualified was diluted, in addition to NBLK, the analyst must also attach the qualifier NBLKd which includes the sample raw result.

Note: Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the diluent and the standard ammonia solutions should approximate that of the samples.

10.4 Calibration Standard

- 10.4.1 Stock: Ammonium chloride (NH₄Cl)
- 10.4.2 Intermediate Standard 1000 ppm:
- 10.4.2.1 Dehydrate Ammonium Chloride (NH₄Cl) in a 105°C oven.
- 10.4.2.2 Allow salt to cool in a dessicator. Weigh out 3.819 g NH₄Cl.

- 10.4.2.3 Dilute to 1 liter with nanopure water in a volumetric flask containing 1.5 mL sulfuric acid.
- 10.4.2.4 Pour the solution into a 1 liter amber bottle. Keep at room temperature, out of sunlight. This standard may be kept for up to 1 year or replaced sooner if comparisons with QC samples indicate a problem.
- 10.4.3 Intermediate standard (50 ppm): Dilute 2.5 mL of 1000 ppm stock standard into 50 mL of diluent. This solution is stored at 4°C up to 28 days.
- 10.4.4 Dilute to make the following calibration standards:
 - 10.4.4.1 2.0 mg/L standard: 2 mL of 50 ppm Intermediate into 50 mL of diluent.
 - 10.4.4.2 The instrument dilutes the 2 ppm standard to the following concentrations: 0.02, 0.05, 0.1, 0.4, 0.8, and 1.6 ppm.
 - 10.4.4.3 Calibration Blank: diluent.

Note: These solutions are stored at 4°C for up to two weeks.

- 10.5 ICV and CCV: A second stock source of NH₄Cl is purchased. An intermediate standard is prepared as specified in section 10.4.2 and section 10.4.3. Working standards are stored at 4°C for up to two weeks. Solutions are prepared as follows:
 - 10.5.1 1.5 mg/L standard: 1.5 mL of 50 ppm Intermediate into 50 mL of diluent. Standard ran at the end of the run.
 - 10.5.2 1.0 mg/L standard: 1 mL of 50 ppm Intermediate into 50 mL of diluent. Standard ran at the beginning, every 10 samples, and at the end of the run.
- 10.6 A calibration blank is analyzed at the beginning of the run, every 10 samples and at the end of the run. *Results must be less than but not more negative than the RL.*
- 10.7 A standard at the reporting limit is analyzed at the end of the run. This is used to validate samples reported as none detected and to evaluate instrument sensitivity should calibration checks show a low bias. This standard should have a signal greater than the method blank.

11.0 PROCEDURE

11.1 Sample Preparation

11.1.1 pH Verification

- 11.1.1.1 Samples that are received preserved are checked for proper pH by the analyst just prior to analysis.
 - 11.1.1.1.1 Place a drop of sample onto a pH strip.
 - 11.1.1.1.2 If the strip is red, the sample is pH<2. Place a checkmark ✓ in the pH column on the prep sheet.

- 11.1.1.1.3 If more preservative is required, add additional sulfuric acid and place a ++ on the prep sheet. Add the qualifier (N_pAdI) when the result is entered.
- 11.1.1.2 The analyst does not need to verify pH of samples that are preserved by the splitter. To indicate this, place a dash – on the prep sheet in lieu of a checkmark.
- 11.1.2 Distillation is required for all samples under the NPDES permit program. First check the distillation comparison study records. If a sample from the same site has been distilled in the past, and the RPD between the distilled and undistilled results is less than or equal to 20, then the sample does not need to be distilled. Raw results ≤ 1.0 mg/L will not generate useful RPD statistics. Instead these results must be within 0.1 mg/L of each other to eliminate the distillation step in the future. If a sample site is not in the study then it must be distilled and added to the study.
 - 11.1.2.1 If the apparatus sits idle for an extended period of time or if samples have left a residue clean the apparatus as follows:
 - 11.1.2.1.1 Rinse all glassware with D.I.
 - 11.1.2.1.2 Make clean out solution by adding 25 mL of borate buffer to 500 mL of D.I. Adjust pH to 9.5 with 6N NaOH solution. Fine tune with 1N NaOH.
 - 11.1.2.1.3 Fill distillation tubes to the line with clean out solution. Add boiling chips.
 - 11.1.2.1.4 Distill over at least 30 mL
 - 11.1.2.1.5 Re-rinse all glassware.
 - 11.1.2.2 Then add 50 mL of sample, LCS, MS, MSD and diluent method blank to individual distillation tubes.
 - 11.1.2.3 Add 2.5 mL of borate buffer to each flask.
 - 11.1.2.4 Adjust to pH 9.5 with 1N or 6N NaOH.
 - 11.1.2.5 Add several porous VWR boiling chips.
 - 11.1.2.6 Distill at 190 °C into a receiving tube containing 5 mL of 0.04N sulfuric acid. Ensure that the tip of the delivery tube is placed below the sulfuric acid level.
 - 11.1.2.7 Collect 50 mL of distillate. Note: Collect at least 30 mL then adjust to 50 mL with D.I. water.
 - 11.1.2.8 Move the delivery tube out of the distillate; add extra water to the distillation tube to prevent dryness until the unit is turned off.
 - 11.1.2.9 Turn off unit when the last sample is finished.
 - 11.1.2.10 Pour distillate into SEAL tubes.
- 11.1.3 Undistilled samples
 - 11.1.3.1 Filter samples if turbid through 0.45 micron microdisk filters directly into SEAL test tubes unless a dilution is made. If a dilution is required, dilute sample, bring up to 50 mL, and filter prior to pouring into SEAL tube. If less than 5 mL of sample is used, dilute with diluent otherwise D.I. water may be used.
 - 11.1.3.2 Use the following volumes based on sample matrix:

- 11.1.3.2.1 Industrial or Influent Wastewater – 2-5 mL.
- 11.1.3.2.2 Effluent Wastewater – 25-50 mL.
- 11.1.3.2.3 Well water - 50 mL.
- 11.1.3.2.4 Solids – Make a 1:10 water extract, extract and swirl periodically for one hour. Filter prior to analysis.

11.2 Calibration

- 11.2.1 The instrument is calibrated every run, when a major instrumentation change is made, or when the calibration check fails.
- 11.2.2 The analyst first analyzes a 1.0 mg/L standard. If the initial calibration check (ICV) is valid, within 90 – 110% of the expected value, a new calibration is not necessary.
- 11.2.3 If the ICV is out, calibrate the instrument. Although a 3-point curve is required, a 7-point curve plus blank is usually prepared. An acceptable curve yields an $r = 0.995$ or better.
- 11.2.4 Calibration Check Standard
 - 11.2.4.1 Prepare a 1.0 ppm standard to check the calibration. Analyze prior to sample analysis, every 10 sample, and at the end of the run to prove that the existing calibration is still valid. A 1.5 ppm standard is analyzed at the end of the run. Recovery must be 90-110%.
- 11.2.5 Calibration Check Blank
 - 11.2.5.1 Analyze a calibration blank prior to sample analysis, every 10 samples, and at the end of the run to prove that the existing calibration is still valid. *Results must be less but not more negative than the reporting limit.*
- 11.2.6 A standard at the reporting limit is analyzed at the end of the run. This is used to evaluate instrument sensitivity should calibration checks show a low bias. This standard should have a signal greater than the method blank

11.3 Sample Analysis By SEAL

- 11.3.1 Allow instrument to warm up
- 11.3.2 Load standard, reagents and samples.
- 11.3.3 See SEAL SOP for general operating instructions.
- 11.3.4 Choose method and begin analysis.
- 11.3.5 When an acceptable calibration has been performed, submit the tray of samples.
- 11.3.6 If diluted samples read below 0.1 mg/L, re-analyze using more sample and diluting to a final volume of 50 mL. Ideally, diluted samples should not fall in the lower quarter of the calibration curve.
- 11.3.7 If any sample reads above 2.0 mg/L, re-analyze using less sample.
- 11.3.8 Watch for possible carry over. Studies have shown that carry over may occur over a concentration of 50 mg/L. Rerun samples at or above the reporting limit following a sample at this concentration. Be mindful of carry over that may happen in the distillation apparatus.

11.4 Calculations

- 11.4.1 Enter raw results into the LIMS. Preparation aliquots are entered as the initial and final volumes. Dilution factors performed after preparation are entered in the DIL column in LIMS. The preparation time is set as the time the last sample in the batch was distilled or filtered. The analysis time is entered individually by the instrument during data transfer.
- 11.4.2 Aqueous reporting limit is 0.1 mg/L, Nonaqueous reporting limit is 1 mg/L. Refer to Element for MDL information.
- 11.4.3 Report 2 significant figures for samples and 3 significant figures for QC samples.
- 11.4.4 Inorganic Nitrogen = $\text{NH}_3\text{N} + \text{NO}_3\text{N} + \text{NO}_2\text{N}$

12.0 CORRECTIVE ACTION FOR OUT OF CONTROL OR UNACCETABLE DATA

- 12.1 See Technical Corrective Action SOP.

13.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 13.1 Refer to "Hazard Communication section of the Chemical Hygiene Plan".

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL)

- 14.1.1 The MDL is the lowest concentration that can be detected for a given analytical method and sample matrix with 99% confidence that the analyte is present. The MDL is determined according to the laboratory's MDL procedure in Appendix H of the QA Manual. MDLs reflect a calculated (statistical) value determined under ideal laboratory conditions in a clean matrix, and may not be achievable in all environmental matrices. The laboratory maintains MDL studies for analyses performed; these are verified at least annually unless method requirements require a greater frequency.

14.2 Limit of Detection (LOD)

- 14.2.1 On an annual basis perform the LOD verification check. Spike a QC sample of reagent water at a level of no more than 2-3 times analyte MDL. Analyte response must be greater than method blank response.

14.3 Initial Demonstration of Capability (IDOC)

- 14.3.1 Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration 10 times the MDL to midrange of the curve. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 20.

14.4 Demonstration of Continuing Proficiency (DOCP)

- 14.4.1 On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four

replicates must be less than or equal to 20%. Performance Evaluation Studies are performed *twice* a year.

15.0 REFERENCES

- 15.1 Standard Methods for the Examination of Water and Wastewater APHA, AWWA, WEF 22nd Edition 4500-NH₃ G
- 15.2 SEAL Analytical AQ2 Method No: EPA 103-A Rev 4 © 6/01/05
- 15.3 EPA Method 350.1. Methods for the Chemical Analysis of Waters and Wastes.
- 15.4 SEAL Analytical AQ2 Operations Manual

16.0 METHOD VARIATIONS

- 16.1 All *italicized items* are an indication of a variation from the method.

17.0 TABLES AND FIGURES

- 17.1 RESERVED

T-316-MBAS

Methylene Blue Active Substances (MBAS)

Standard Operating Procedure

SM 5540 C

Effective Date: 12/18/2015

Revision#: 1.0

Approved by: _____

(signature/date)

Meets Quality System Requirements: _____

(signature/date)



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1.0 SCOPE AND APPLICATION

- 1.1 Anionic surfactants are the most prominent substances that are determined as MBAS. This test measures the content of these in drinking water and wastewater plus other substances that react with Methylene blue under the conditions of the test. Linear alkylbenzene sulfonate (LAS) is the standard to which the test is compared. This method may also be used for solid samples if both client and regulator agree to this variation.
- 1.2 Environmental Relevance (5540)
 - 1.2.1 Surfactants enter waters and wastewaters mainly by discharge of aqueous wastes from household and industrial laundering and other cleansing operations. A surfactant combines in a single molecule a strongly hydrophobic group with a strongly hydrophilic one. Such molecules tend to congregate at the interfaces between the aqueous medium and the other phases of the system such as air, oily liquids, and particles, thus imparting properties such as foaming, emulsifications, and particle suspension.
 - 1.2.2 Anionic surfactant responses: Soaps do not respond in the MBAS method. Those used in or as detergents are alkali salts of C10-20 fatty acids $[RCO_2]^-Na^+$, and though anionic in nature they are so weakly ionized that an extractable ion pair is not formed under the conditions of the test. Non-soap anionic surfactants commonly used in detergent formulations are strongly responsive. These include principally surfactants of the sulfonate type $[RSO_3]^-Na^+$, the sulfate ester type $[ROSO_3]^-Na^+$, and sulfated nonionics $[REnOSO_3]^-Na^+$. They are recovered almost completely by a single $CHCl_3$ extraction.
- 1.3 Reporting Limit: 0.08 mg/L
- 1.4 Working Range
 - 1.4.1 The working range for this analysis is 0.08 to 0.75 mg/L.
 - 1.4.2 The maximum contaminant limit for drinking waters is 0.5 mg/L.

2.0 SUMMARY OF METHOD

- 2.1 Methylene blue reacts in an aqueous solution to form an immiscible organic liquid. It comprises three successive extractions from acid aqueous medium containing excess methylene blue into chloroform, followed by an aqueous backwash. The intensity of the blue color upon extraction into Chloroform is a measure of the MBAS concentration by spectrophotometry at 652 nm.

3.0 DEFINITIONS- See Definitions Appendix in Quality Manual

4.0 SAFETY

- 4.1 Identified Hazards
 - 4.1.1 Due to presence of Chloroform, extractions must be performed in a hood. The Department of Health and Human Services (DHHS) has determined that chloroform may reasonably be anticipated to be a carcinogen. Avoid contact with skin or breathing of vapor. General laboratory safety practices apply. (Refer to Chemical Hygiene Plan.)

- 4.2 SDS Review: Safety Data Sheets are available for items listed under section 8.0 if applicable
 - 4.3 Required PPE: Employees are required to wear a lab coat, safety glasses, and appropriate gloves when working in the lab or handling samples, standards, acids or bases.
 - 4.4 Required training: Review of safety data sheets, PPE, and fume hood training
- Safety precautions noted throughout procedure are noted as **SAFETY NOTES**.

5.0 INTERFERENCES

- 5.1 Make sure glassware that holds or delivers solvent is dry.
- 5.2 Negative interferences may occur due to cationic materials present in solution that interfere with the reaction (i.e. cationic surfactants or amines that compete with methylene blue in formation of ion pairs). These may cause recoveries to be incomplete and variable.
- 5.3 Sulfides may react with Methylene blue to form a colorless product, making analysis impossible. Eliminate this interference by adding a few drops of 30% hydrogen peroxide.
- 5.4 Samples containing large amounts of solid material should be diluted. Extra methylene blue should be added to replace reagent that has been absorbed onto the particles.
- 5.5 Chlorides at concentration of about 1000 mg/L show a positive interference but the degree of interference has not been quantified. For this reason dilute samples containing high chloride concentrations prior to analysis.
- 5.6 The types of interferences discussed in the method are highly unlikely to be present in drinking water samples. For wastewater samples, if the level of MBAS determined under our normal procedure is close to the critical level and the client or regulator has a concern, additional steps will be taken to deal with interferences.
- 5.7 Because of inherent properties of surfactants, special analytical precautions are necessary. Avoid foam formation because the surfactant concentration is higher in the foam phase than in the associated bulk aqueous phase and the latter may be significantly depleted. Invert samples and standards instead of shaking. If foam is formed, let it subside by standing, or collapse it by other appropriate means, and remix the liquid phase before sampling.
- 5.8 If color poses an interference, a background reading is necessary. Perform the entire analysis without the methylene blue reagent. Subtract this background absorbance from the sample absorbance, prior to converting the result to concentration.

6.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

- 6.1 RESERVED

7.0 EQUIPMENT AND SUPPLIES

- 7.1 Spectrophotometer: Spectronic Genesys 10uv for use at 652 nm providing a light path of 1 cm or longer.
- 7.2 Separatory Funnels: Teflon or glass, 500 mL or larger.
- 7.3 Graduated Cylinders and Glass Pipets: varying sizes
- 7.4 Beakers
- 7.5 *Untreated* Glass wool
- 7.6 Drying funnels
- 7.7 Pall Life Sciences Type A/E glass fiber filter disk (47mm); Pall Life Sciences Supor – 450 Membrane Filter 47mm)

Note: All glassware is cleaned immediately after use by thorough rinsing with three portions of D.I. water. If glassware still appears dirty from sample, 1:1 HCl rinse is used to remove blue color that may remain in glassware. Glassware is always finished with a final D.I. rinse.

8.0 REAGENTS AND STANDARDS

- 8.1 Methylene Blue: Add 100 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 13.6 ml concentrated Sulfuric Acid, and 0.06 g Methylene Blue to a 1 liter volumetric flask. Bring up to volume with deionized water, pour into a 2 L bottle and add an additional liter of deionized water.
 - 8.2 Wash solution: Add 100g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 13.6 ml concentrated Sulfuric Acid to a 2 L bottle and dilute to volume with deionized water.
 - 8.3 0.5 N Sodium Hydroxide: Dissolve 10 g NaOH in approximately 150 mL deionized water. Let cool. Dilute to 500 ml with deionized water.
 - 8.4 0.5 N Sulfuric Acid: Add 7 ml of concentrated H_2SO_4 to approximately 150mL of deionized water. Dilute to 500 ml with deionized water.
 - 8.5 Phenolphthalein indicator - Dissolve 1 g of Phenolphthalein in 100 ml of reagent alcohol: ethyl, isopropyl, or n-propanol. Add 100 ml D.I. water.
- Note: Above reagents are stored up to 6 months at room temperature.
- 8.6 Isopropyl alcohol - Expires 1 year from open date and stored in flame-resistant cabinets.
 - 8.7 Chloroform - Fresh and reclaimed expires 2 years from open date.
 - 8.7.1 Use a label for the reclaimed bottle that includes the original chloroform vendor, lot#, opened date, and expiration date. Add a "reclaimed date" as it changes. Add vendor, lot#, and opened date whenever a new bottle is

added to the reclaimed collection bottle. Start a new reclaimed collection bottle when you are close to the original 2 year expiration date.

8.8 Reagent water: D.I.

8.9 LAS Stock Standards:

8.9.1 Stock standards are purchased from a certified manufacturer at a concentration of 1000ppm. Solutions are stored in sealed vials, protected from light, at 4°C. Once the stock vial is opened, it may be kept up to 12 months as long as this does not exceed the manufacturer expiration date. Do not mix contents of individual vials. Stock standards can be replaced sooner if comparisons with QC samples indicate a problem.

8.9.1.1 Source 1 – For calibration standards

8.9.1.2 Source 2 – For LCS standards (a second lot number)

8.10 LAS Intermediate Standards:

8.10.1 Source 1 Calibration Intermediate Std. *50ppm*: Pipette 5 ml source 1 stock standard (1000 mg/L) into a 100 milliliter volumetric and dilute to 100 ml with deionized water. Store in the refrigerator for up to *6 months*. Solutions will be replaced sooner if comparisons with QC samples indicate a problem. This standard will be used for curve check.

8.11 Source 2 LCS Intermediate Std. *40ppm*: Pipette 4 ml of source 2 stock standard and dilute to 100 ml with deionized water. Store in the refrigerator for up to *6 months*. Solutions will be replaced sooner if comparisons with QC samples indicate a problem. This standard will be used for the Lab Control and spikes.

9.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

9.1 Sample may be collected in either plastic or glass.

9.2 Store unpreserved at 4°C +/- 2 °C.

9.3 Analyze within 48 hours from sampling.

10.0 QUALITY CONTROL

10.1 A laboratory control sample (LCS) is analyzed every 20 samples or once per batch whichever is more and reported per matrix type. It must meet acceptance criteria for batch acceptance. Acceptance limits are generated from historical data. See LIMS for most current limits. If QC does not meet acceptance criteria, the cause must be determined before the analyst may proceed.

10.2 A Matrix Spike (MS) and Matrix Spike Duplicate (MSD) are analyzed for every 20 samples per matrix type. If they do not fall within the recommended range, troubleshooting is performed to determine the cause. The analysis is not controlled on the MS and MSD. Acceptance limits are generated from historical data. See LIMS for most current limits. The maximum RPD is set at 20.

10.3 A method blank is analyzed every 20 samples or once per batch whichever is more and reported per matrix type. Results must read less than or equal to one-

half the reporting limit. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than 1/10 the measured raw concentration of the sample. Blank results below the reporting limit are considered to be ND and will not require a note.

11.0 PROCEDURE

11.1 Calibration

- 11.1.1 A minimum of five calibration standards, including the origin, are required by the method. Currently, calibration standards are prepared at ten concentration levels covering the referenced working range, including zero, and analyzed on a yearly basis.
- 11.1.2 The current curve was made using the following calibration standards from the 50mg/L Source 1 Int. Standard (Section 8.10.1).
 - 11.1.2.1 0.08mg/L 400ul in 250mL
 - 11.1.2.2 0.20mg/L 1mL in 250mL
 - 11.1.2.3 0.40mg/L 2mL in 250mL
 - 11.1.2.4 0.60mg/L 3mL in 250mL
 - 11.1.2.5 0.80mg/L 4mL in 250mL
- 11.1.3 An acceptable curve yields an $r^2 = 0.99$ or better.
- 11.1.4 A regression equation is generated from the curve specifying the molecular weight of the LAS (listed on the C of A) used.
- 11.1.5 Results are calculated from the regression equation. Results may not be reported over the high standard. Samples may be diluted to obtain results within the calibration range.
- 11.1.6 Immediately following calibration a 0.32 mg/L BS is analyzed using the LCS source to verify the curve. Recovery must be within established historical criteria or a new curve may be necessary.
- 11.1.7 Continuing Calibration Verification Checks: The calibration is verified on a daily basis by analysis of the Lab Control, which is at the beginning of the day's batch. Every 20 samples or at the end of the day's batch(es), the Calibration Check Standard is analyzed. Acceptance limits are generated from *historical data*. See LIMS for most current limits.
- 11.1.8 An RL check is analyzed at the end of each run. *Recovery should be within 50-150%.*

11.2 Sample Analysis

- 11.2.1 Analyze a 250 ml aliquot of sample when results are expected to be between 0.08mg/L and 0.4mg/L. Less sample may be used if the result is estimated to be higher and depending on the amount of turbidity and/or foaminess of the sample. Bring volume up to 250 mL with D.I. Do not over dilute samples. A sample reading should not be in the lower 25% of the calibration curve prior to applying a dilution factor. If a smaller aliquot of sample is taken due to sample availability or matrix, the reporting limit must be adjusted.
- 11.2.2 Pour sample into separatory funnel.
- 11.2.3 Adjust pH. Add 2 drops of phenolphthalein indicator. Add 0.5 N NaOH until the sample turns pink. (If the sample is faint pink, the addition of sulfuric acid is not needed). Add 0.5 N H₂SO₄ until the sample just turns

- clear again. If the sample turns pink immediately upon adding the indicator, then do not add NaOH.
- 11.2.4 Add 25 ml of methylene blue reagent.
 - 11.2.5 Add approx. 10 ml of chloroform using the estimated mark on the funnel.
 - 11.2.6 Cap the funnel and shake in a half moon fashion for 30 seconds and let phases separate.
 - 11.2.6.1 If the sample forms a large emulsion, try one of the following options:
 - 11.2.6.1.1 Drain emulsion onto glass wool. Rinse and change glass wool prior to wash.
 - 11.2.6.1.2 Use less sample. This must however produce a reportable result.
 - 11.2.6.1.3 Start with the original sample aliquot and add approximately 5 mL of Isopropyl alcohol to break up the emulsion. If the Isopropyl alcohol is added to any sample, then the same amount of Isopropyl alcohol must be added to the method blank, lab control, and any related spikes.
 - 11.2.6.1.4 See manager or experienced analyst for guidance.
 - 11.2.7 Before draining chloroform layer, swirl gently, then let settle. Some samples require a longer period of phase separation than others.
 - 11.2.8 Filter the bottom layer of chloroform through glass wool and into a 50 ml dry graduated cylinder.

Note: Do not pack too much glass wool tightly into funnel as it may trap methylene blue.

- 11.2.9 Do steps 11.2.5 – 11.2.6 two more times.
- 11.2.10 If blue color in water phase becomes faint or disappears, discard and repeat using a smaller amount of sample.
- 11.2.11 After 3 extractions, rinse the glass wool with a small amount of chloroform and squeeze with tongs. Change the glass wool if it contains water or is visibly dirty or contaminated.
- 11.2.12 Throw the sample remaining in the separatory funnel into the waste bottle.
- 11.2.13 Pour the chloroform phase from graduated cylinder into the separatory funnel.
- 11.2.14 Add 50 ml of wash solution into the funnel. Shake for 30 seconds in a half moon fashion and let phases separate. Before draining chloroform layer, swirl gently, then let settle. Filter thru glass wool.
- 11.2.15 Pour approximately 5 ml of chloroform into the funnel. Shake 30 seconds and filter.
- 11.2.16 Repeat step 11.2.15. Swirl funnel and ensure that all the chloroform has settled out of the aqueous phase. Discard the sample remaining in the separatory funnel into the waste bottle.
- 11.2.17 Rinse the glass wool with chloroform.
- 11.2.18 Bring the cylinder up to 50 ml with chloroform.
- 11.2.19 Pour the chloroform extract into a dry 100 ml beaker to homogenize the sample.
- 11.2.20 Make sure the spec has been on for at least 20 minutes. Set the spec to read at 652 nm. Then add a cuvette with clean chloroform and set the Absorbance to 0.
- 11.2.21 Pour sample into cuvette and read the absorbance.

- 11.2.22 Record the result on the data page.
- 11.2.23 Watch for possible carry over. Studies have shown that carry over may occur over a concentration of 0.75 mg/L. If a sample is at or above this concentration, thoroughly rinse 50 ml graduated cylinder and cuvette with chloroform prior to the next sample.

11.3 Calibration Check Standard:

- 11.3.1 CCV (0.6 mg/L): Pipet 3 mL of Source 1 Intermediate standard into 250mL. Analyze 250 mL.
- 11.3.2 Follow the steps after 11.2 in the procedure above.

11.4 Lab Control Sample (0.32 mg/L)

- 11.4.1 LCS: Pipet 2 mL of Source 2 Intermediate standard into 250 mL. Analyze 250 mL.
- 11.4.2 Follow Steps after 11.2 in the procedure above.

11.5 Spikes (0.16 mg/L):

- 11.5.1 Choose a variety of samples.
- 11.5.2 Use the same dilution of sample for the spike. Spike sample with 1ml of source 2 intermediate standard into the separatory funnel.
- 11.5.3 Follow steps after 11.2 in the procedure above.

11.6 Solid samples

- 11.6.1 Make a 1:10 water extract. (For example: Use 25 grams of sample and 250 ml of D.I. water.)
- 11.6.2 Swirling every so often, allow sample to extract for 1 hour. Filter extract through a 0.45 micron membrane type filter.
- 11.6.3 Take a 100 mL aliquot of extract, unless the sample is known to be high in surfactants. Follow steps 11.2 thru 11.2.23 using extra methylene blue reagent.
- 11.6.4 Report results back to the sample.

11.7 Method Blank

- 11.7.1 Analyze 250mL of D.I. water.

11.8 Calculations

- 11.8.1 Results are calculated from a calibration curve based on LAS standards with a molecular weight of 320 g/mole.
- 11.8.2 Convert Absorbance to concentration by plugging the spectrophotometer reading into the regression equation.
- 11.8.3 Do not report results higher than the highest calibrator.

11.9 Reporting

- 11.9.1 Enter raw result and dilution factor into LIMS.
- 11.9.2 Report concentrations as *mg/L*.

- 11.9.3 The reporting limit of this analysis is 0.08 mg/L and the most recent method detection limit is listed in Element.
- 11.9.4 Analyzed date and time is documented as the date and time the sample was extracted.
- 11.9.5 Set the prepared time to equal the analyzed time in Element.
- 11.9.6 When a diluted sample is ND, the sample must be qualified with N_RLm.
- 11.9.7 When a diluted sample is ND due to limited volume, the sample must be qualified with N_RLv.

12.0 CORRECTIVE ACTION FOR OUT OF CONTROL OR UNACCETABLE DATA

- 12.1 See Technical Corrective Action SOP.

13.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 13.1 Refer also to "Hazard Communication section of the Chemical Hygiene Plan".
- 13.2 Sample remaining in the separatory funnel is neutralized and dumped in the 30 gallon drum.
- 13.3 In an attempt to minimize the amount of chloroform disposal, the chloroform extract waste is collected for reclamation. Reclaimed chloroform bottles must have reclaim date and initials.

14.0 METHOD PERFORMANCE

- 14.1 Method Detection Limit (MDL)

- 14.1.1 The MDL is the lowest concentration that can be detected for a given analytical method and sample matrix with 99% confidence that the analyte is present. The MDL is determined according to the laboratory's MDL procedure in Appendix H of the QA Manual. MDLs reflect a calculated (statistical) value determined under ideal laboratory conditions in a clean matrix, and may not be achievable in all environmental matrices. The laboratory maintains MDL studies for analyses performed; these are verified at least annually unless method requirements require a greater frequency.

- 14.2 Limit of Detection (LOD)

- 14.2.1 On a yearly basis, an aliquot of water is spiked at a level of not more than 2-3 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.

- 14.3 Initial Demonstration of Capability (IDOC)

- 14.3.1 Prior to analysis of samples or when a significant change is made to the method, an Initial Demonstration of Capability Study is performed. This is accomplished by analysis of four replicates of a QC sample made at a concentration between 10 times the MDL and the midpoint of the calibration curve. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 20.

14.4 Demonstration of Continuing Proficiency (DOCP)

- 14.4.1 On an annual basis, analyst must turn in valid LCS data from four consecutive LCS samples or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%. Performance Evaluation Studies are performed twice a year.

15.0 REFERENCES

- 15.1 Standard Methods for the Examination of Water and Wastewater: APHA/AWWA/WPCF, 22nd edition. 5540C
- 15.2 Genesys 10 Operator's Manual. Thermo Electron Corporation

16.0 METHOD VARIATIONS

- 16.1 All *italicized items* are an indication of a variation from the method.

17.0 TABLES AND FIGURES

- 17.1 RESERVED

Sample Preparation for Metals Analysis

Sample Preparation for Metal Analysis

Standard Operating Procedure

Effective Date: 12/17/2015

Revision#: 1.0

Approved by: _____
(signature/date)

Meets Quality System Requirements: _____
(signature/date)



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1.0 SAMPLE PREPARATION SUMMARY:

- 1.1 To preserve for trace metals, acidify the sample with 1:1 nitric acid to a pH of less than 2 units. Use no more than 6 ml acid per quart of sample (3%).
- 1.2 Samples for trace metal analysis are usually prepared by digestion. Digestion is a process where metallic elements are converted into soluble salts by reaction with acids or bases. Some samples may require dilution, filtration, or centrifugation prior to or after digestion so they are ready for analysis.
- 1.3 The digestion procedure is selected as follows:
 - 1.3.1 **Drinking waters and Wastewaters**
 - 1.3.1.1 The sample is received by the analyst who examines the sample.
 - 1.3.1.2 If the sample is clear with a turbidity reading <1 NTU then the sample is analyzed as received – It is simply acidified with 2% HNO₃/1%HCl and analyzed undigested.
 - 1.3.1.3 If there is inorganic residue in the sample, turbidity reading >1 NTU then the sample is digested by hot block - EPA method 200.2 (SOP M02C).
 - 1.3.1.3.1 Most metals: 2% HNO₃/1%HCl
 - 1.3.1.3.2 Drinking water chromium, vanadium, low ML's (see section 2.3): 2%HNO₃ only.
 - 1.3.2 **UCMR3**
 - 1.3.2.1 See Section 11.1.3 of M02C
 - 1.3.3 **Ground waters**
 - 1.3.3.1 The sample is digested by hot block 2% HNO₃/1%HCl- EPA method 200.2 (SOP M02C).
 - 1.3.4 **Solid or sludge (non petroleum based)**
 - 1.3.4.1 The sample is digested by hot block 20% HNO₃/10%HCl-EPA method 3050B (SOP M02C).
 - 1.3.4.2 Solid results are reported as received.
 - 1.3.4.3 Sludge results are calculated on a dry weight basis using the Total Solid value obtained separately.
 - 1.3.5 Samples with high organic content (i.e. **petroleum based**) can be dissolved using EPA 3040 (for example with organic lead). This method does not yield total results. See section 2.1.
 - 1.3.6 **Oils** can also be digested using the ASTM wet ash method. See section 2.2.
 - 1.3.7 For cold vapor Mercury analyses, use SM 3112B for waters or wastewaters and EPA Method 7470 for groundwaters and extracts-(SOP M06). Use EPA Method 7471A (SOP M06A) for solid or semisolid hazardous waste and sludges.

2.0 DISSOLUTION/DIGESTION METHODS.

2.1 EPA method 3040:

- 2.1.1 Weigh out a 2 g representative sample of the waste or extract. Separate and weigh the phases if more than one phase is present.
- 2.1.2 Dilute the aliquot with appropriate solvent (either MIBK or Xylene).
- 2.1.3 Samples must be analyzed by the method of standard additions.

Note: EPA Method 3040 is only used by our laboratory per client request.

2.2 ASTM D1548-63 – Wet Ash Procedure for Oil.

- 2.2.1 Weigh 2g of sample into a ceramic dish with a handle.
- 2.2.2 Slowly add 2.5 mL concentrated H_2SO_4 .
- 2.2.3 Heat on a hotplate, stirring occasionally with a stir rod until white fumes form indicating that moisture is gone. CAUTION: If the sample is heated too fast or not stirred enough, splattering may occur. Partially cover with a watch glass to prevent loss of sample.
- 2.2.4 Continue heating until white fumes stop.
- 2.2.5 Cover with a watch glass and place in 525 deg C furnace for 8 hours. Cool.
- 2.2.6 Add 5 - 10 mL NANOpure water.
- 2.2.7 Add 5 mL HNO_3 and 2.5 mL HCl.
- 2.2.8 Heat on hotplate until sample clears.
- 2.2.9 Dilute to 25 mL with NANOpure water.
- 2.2.10 Analyze by ICP or ICPMS.
- 2.2.11 Batch QC consists of a method blank and sample duplicate. (Currently BS, MS, and MSD are analyzed for internal monitoring only.)

2.3 EPA 1638 modified for ML metals (Priority Pollutant Project)

- 2.3.1 Measure 25 mLs of sample into a 50mL red cap tube.
- 2.3.2 Add 0.5 mLs of HNO_3 .
- 2.3.3 Digest on hot plate in beaker of D.I. for 2 hours at 85°C.
- 2.3.4 Analyze without further dilution.
- 2.3.5 Calibrate with standards that are prepared at the ML reporting limit.
- 2.3.6 Normal Batch QC is performed: method blank, blanks spike, matrix spike and matrix spike duplicate.

3.0 SAFETY

3.1 Identified Hazards

- 3.1.1 Concentrated acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.
- 3.1.2 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood if the sample has any indication that there may be reactive materials present (odor, color, consistency, incorrect pH, etc.).

- 3.2 SDS Review Safety Data Sheets are available for items listed under section 8.0 if applicable.

- 3.3 Required PPE Employees are required to wear a lab coat, safety glasses, and appropriate gloves when working in the lab or handling samples, acids or standards.
- 3.4 Required training Required training; Review of safety data sheets, PPE, and fume hood training.

Safety precautions noted throughout procedure are noted as **SAFETY NOTES**.

4.0 INTERFERENCES

- 4.1 In sample preparation, contamination is of prime concern. The work area, including bench top and fume hood, should be periodically cleaned in order to eliminate environmental contamination.
- 4.2 Chemical interferences are matrix dependent and cannot be documented previous to analysis.
- 4.3 Boron and silica from the glassware will grow into the sample solution during and following sample processing. For critical determinations of boron and silica, only quartz and/or PTFE plastic labware should be used. When quartz beakers are not available for extraction of solid samples, to reduce boron contamination, immediately transfer an aliquot of the diluted extract to a plastic centrifuge tube for storage until time of analysis. A series of laboratory reagent blanks can be used to monitor and indicate the contamination effect.

5.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

- 5.1 RESERVED

SOP T-508 200.7

SOP Title

Standard Operating Procedure

EPA Method#: 200.7

Effective Date: 12/17/2015

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(signature/date)

Meets Quality System Requirements: _____

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1.0 SCOPE AND APPLICATION

- 1.1 Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is used to determine metals and some nonmetals in solution.
- 1.2 This method is a consolidation of existing methods for water, and wastewater.
- 1.3 This method is applicable to the following analytes:

Table 1

<u>ANALYTE:</u>	<u>CAS #</u>	<u>MCL (ppb)</u>
Aluminum	7429-90-5	1000
Antimony	7440-36-0	6
Arsenic	7440-38-2	50
Barium	7440-39-3	1000
Beryllium	7440-41-7	4
Boron*	7440-42-8	--
Cadmium	7440-43-9	5
Calcium	7440-70-2	--
Chromium	7440-47-3	50
Cobalt	7440-48-4	--
Copper	7440-50-8	1000
Iron	7439-89-6	300
Lead	7439-92-1	15
(Lithium)	7439-93-2	--
Magnesium	7439-95-4	--
Manganese	7439-96-5	50
(Mercury)	7439-97-6	2
Molybdenum	7439-98-7	--
Nickel	7440 02-0	100
Potassium	7440-09-7	--
Selenium	7782-49-2	50
Silica	7631-86-9	--
Silver	7440-22-4	100
Sodium	7440-23-5	--
(Strontium)	7440-24-6	--
Thallium	7440-28-0	2
Tin	7440-31-5	--
(Titanium)	7440-32-6	--
Vanadium	7440-62-2	--
Zinc	7440-66-6	5000

Total Hardness

Note: Analytes in parenthesis are not certified by NELAP.

* ELAP Certified

- 1.4 INSTRUMENTATION: Perkin Elmer Optima 5300DV

- 1.5 For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)] consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NPDES, and Part 141 141.23 for drinking water), and the latest Federal Register announcements.
- 1.6 ICP-AES can be used to determine dissolved analytes in aqueous samples after suitable filtration and acid preservation. To reduce potential interferences, dissolved solids should be < 2000 mg/L (Sect. 4.2).
- 1.7 Samples may be analyzed directly by pneumatic nebulization without acid digestion if the sample has been properly preserved with acid and has turbidity of < 1 NTU at the time of analysis. This total recoverable determination procedure is referred to as "direct analysis". This method is not sensitive enough to determine some drinking water analytes without preconcentration. The preferred method for those analytes is ICP-MS
- 1.8 For the determination of total recoverable analytes in aqueous samples a digestion is required prior to analysis when the elements are not in solution (e.g., aqueous samples that may contain particulate and suspended solids). See SOP for digestion procedures.
- 1.9 When determining boron and silica in aqueous samples, only plastic, PTFE or quartz labware *should be* used from time of sample collection to completion of analysis. Borosilicate glass is avoided to prevent contamination of these analytes.
- 1.10 Detection limits and linear ranges for the elements will vary with the wavelength selected, the spectrometer, and the matrices. Table 2 provides estimated instrument detection limits for the listed wavelengths. However, actual method detection limits and linear working ranges will be dependent on the sample matrix, instrumentation, and selected operating conditions.
- 1.11 Working Ranges: Reporting limit to 90% of Linear Dynamic Range Maximum. See LIMS for most current RL and MDL.

2.0 SUMMARY OF METHOD

- 2.1 An aliquot of a well mixed, homogeneous aqueous sample is accurately weighed or measured for sample processing. For the determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is < 1 NTU, the sample is made ready for analysis by the appropriate addition of nitric acid and hydrochloric acid.
- 2.2 The analysis described in this method involves multielemental determinations by ICP-AES. The instrument measures characteristic atomic-line emission spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element specific emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the line spectra are monitored at specific wavelengths by a photosensitive device. Photocurrents from the photosensitive device are processed and controlled by a computer system. A background correction technique compensates for variable background

contribution to the determination of the analytes. Background is measured adjacent to the analyte wavelength during analysis. Interferences are more completely discussed in section 5.0.

- 2.3 The plasma converts metals in the sample from complex molecules to their atomic form and provides energy to excite them. Each metal will absorb energy at a specific wavelength. Eventually the metal will return to its ground state by emitting the energy it absorbed. The ICP uses this emission of energy to quantify each metal. Each metal emits light at a different wavelength. This is called polychromatic. These wavelengths are separated by a monochromator and detected by a photomultiplier tube.

3.0 DEFINITIONS- See Definitions Appendix in Quality Manual

4.0 SAFETY

4.1 Identified Hazards

- 4.1.1 Concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.
- 4.1.2 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood if the sample has any indication that there may be reactive materials present (odor, color, consistency, incorrect pH, etc.).
- 4.1.3 Analytical plasma sources emit radio frequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.

- 4.2 SDS Review Safety Data Sheets are available for items listed under section 8.0 if applicable.

- 4.3 Required PPE; Employees are required to wear a lab coat, safety glasses, and appropriate gloves when working in the lab or handling samples, acids or standards.

- 4.4 Required training; Review of safety data sheets, PPE, and fume hood training.

Safety precautions noted throughout procedure are noted as **SAFETY NOTES**.

5.0 INTERFERENCES

- 5.1 Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, or unresolved overlap of molecular band spectra.

- 5.1.1 Background emission and stray light are usually compensated for by subtracting the background emission determined by measurement(s)

adjacent to the analyte wavelength peak. Spectral scans of samples or single element solutions in the analyte regions indicate not only when alternate wavelengths are desirable because of severe spectral interference, but also show whether the most appropriate estimate of the background emission is provided by an interpolation from measurements on both sides of the wavelength peak or by the measured emission on one side or the other. The location(s) selected for the measurement of background intensity are determined by the complexity of the spectrum adjacent to the wavelength peak. The selected location(s) used for routine measurement are free of off-line spectral interference (interelement or molecular) or adequately corrected to reflect the same change in background intensity as occurs at the wavelength peak.

5.1.2 Spectral overlaps may be avoided by using an alternate wavelength or can be compensated for by equations that correct for interelement contributions, which involves measuring the interfering elements. On-line spectral interferences observed for the recommended wavelengths are given in Table 3. When operative and uncorrected, these interferences will produce false-positive determinations and be reported as analyte concentrations. The interferences listed are only those that occur between method analytes.

5.1.3 When determining trace analytes, the analyst is aware of potential spectral interferences and makes correction where necessary by calculating the interference factor and utilizing a computerized correction routine or utilizing another wavelength.

5.1.4 The interference effects are evaluated for each individual instrument whether configured as a sequential or simultaneous instrument. Interferences are specific to the instrument and operating conditions. The analyst is required to determine and document for each wavelength the effect from the known interferences given in Table 2 and to check for the presence of interferents in samples and utilizing a computerized correction routine. To determine the appropriate location for off-line background correction, the analyst scans the area on either side adjacent to the wavelength and records the apparent emission intensity from all other method analytes. This spectral information is documented and kept on file. The location selected for background correction is free of off-line interelement spectral interference. If a wavelength other than the recommended wavelength is used, the analyst determines and documents both the on-line and off-line spectral interference effect from all method analytes and provide for their automatic correction on all analyses. Tests to determine the spectral interference are done using analyte concentrations that will adequately describe the interference.

5.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they are reduced by using a peristaltic pump and an internal standard. Additionally the analyst may utilize such means as diluting the sample if the recovery on the internal standard is low. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. This problem is controlled by diluting the sample when necessary. Also, it has been reported that better control of the argon flow rates,

especially for the nebulizer, improves instrument stability and precision; this is accomplished with the use of mass flow controllers.

- 5.3 Chemical interferences include molecular-compound formation, ionization effects, and solute-vaporization effects. Normally, these effects are not significant with the ICP-AES technique. If observed, they can be minimized by careful selection of operating conditions (such as incident power and observation height), by buffering of the sample, by matrix matching, and by standard-addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.
- 5.4 Memory interferences are controlled by the use of a rinse blank between analyses. The instrument automatically increases the rinse time following a high sample. If a memory interference is still suspected, the sample is re-analyzed after the system is free of contamination.
 - 5.4.1 Necessary rinse times for a particular element are estimated upon initial set up of the instrument. This is achieved by aspirating a standard containing elements at a concentration near the top of the linear dynamic range. The aspiration time for this sample should be the same as a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to *less than one half the reporting limit* is applied. If one half the reporting limit is less than the MDL, signals must be less than the MDL.
 - 5.4.2 When there is suspected additional memory interference between samples, the rinse cycle is repeated and the sample is reanalyzed.

6.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

- 6.1 RESERVED

7.0 EQUIPMENT AND SUPPLIES

- 7.1 Inductively coupled plasma emission spectrometer: Perkin Elmer Optima 5300DV Computer-controlled emission spectrometer with background- correction capability.
 - 7.1.1 Radio-frequency generator compliant with FCC regulations per PE manual.
 - 7.1.2 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders.
 - 7.1.3 A variable speed peristaltic pump is required to deliver both standard and sample solutions to the nebulizer. If an Elemental Science FAST system is used to deliver the standards and samples to the nebulizer then the variable speed peristaltic pump is required to deliver both carrier (i.e. rinse water) through the valve and internal standard directly to the nebulizer.
 - 7.1.4 (optional) Mass flow controllers to regulate the argon flow rates, especially the aerosol transport gas, are highly recommended. Their use will provide more exacting control of reproducible plasma conditions.
- 7.2 Autopipetters

- 7.3 Labware - A clean laboratory work area designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by contributing contaminants through surface desorption or leaching, depleting element concentrations through adsorption processes. All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) is sufficiently cleaned for the task objectives. Plastic volumetric flasks are rinsed with 1:1 nitric and NANOpure water. See preparation SOP for washing instructions of digestion glassware.
- 7.3.1 Glassware - Volumetric flasks, graduated cylinders, funnels and centrifuge tubes (metal-free plastic).
 - 7.3.2 Assorted calibrated autopipettes.
 - 7.3.3 Hot block tubes
 - 7.3.4 15 dram snap cap vials.
 - 7.3.5 Plastic autosampler tubes.
 - 7.3.6 One-piece stem FEP wash bottle with screw closure, 125-mL capacity.
- 7.4 Plastic sample bottles: New bottles are used to store samples. Each new lot of bottles is tested for trace contaminants by analysis of an acid blank placed in one sample bottle from that lot. Results must be below the MDL.

8.0 REAGENTS AND STANDARDS

- 8.1 Reagents may contain elemental impurities that might affect analytical data. Only high-purity reagents that conform to the American Chemical Society specifications 13 should be used whenever possible. If the purity of a reagent is in question, analyze for contamination. All acids used for this method must be purchased from the manufacturer intra analyzed (spectro grade).
- 8.2 Hydrochloric acid, concentrated (sp.gr. 1.19) - HCl.
- 8.3 Nitric acid, concentrated (sp.gr. 1.41) - HNO₃.
- 8.4 Reagent water. All references to water in this method refer to ASTM Type I grade water. Our lab uses NANOpure.
- 8.5 Standard Stock Solutions - Stock standards are purchased from a reputable supplier either separately or as mixed solutions. Two sources are acquired to verify instrument performance. Solutions are stored at room temperature. Manufacturer specified holding times are observed.
- 8.6 Working Standard Solutions - Solutions are made from above stock solutions in 1% HCl and 2% HNO₃. Solutions are stored at room temperature for up to six months. Solutions will be replaced sooner if comparison with QC samples indicates a problem. See standard log for recipes.

Note: If the other QC standards indicate a problem with a mixed standard, each stock solution should be analyzed separately to determine the problem. There can be slight high bias of Fe, Al, or other metals in mixed standards since stock sources of some metals contain trace amounts of other metals. If this bias causes QC samples to be out of range, each stock standard will be tested for the bias and this bias will be added to the true value of the metal in the standards

- 8.7 Blanks - Four types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure, the laboratory fortified blank is used to assess routine laboratory performance and a rinse blank is used to flush the instrument uptake system and nebulizer between standards, check solutions, and samples to reduce memory interferences.
- 8.7.1 The calibration blank for aqueous samples and extracts is prepared by acidifying reagent water to the same concentrations of the acids as used for the standards. The calibration blank is stored in a contaminant free plastic bottle.
- 8.7.2 The laboratory reagent blank (LRB) (method blank (MB)) must contain all the reagents in the same volumes as used in the processing of the samples. The LRB is carried through the same entire preparation scheme as the samples including sample digestion, when applicable.
- 8.7.3 The laboratory fortified blank (LFB) [Laboratory Control Sample (LCS) or Blank Spike (BS)] is prepared by fortifying an aliquot of the laboratory reagent blank with all analytes to a suitable concentration (approximately 100 times their respective MDL). 200 μ Ls of an Intermediate standard is spiked into 5 mL of NANOpure water. See batch standard log for individual metal concentrations. The LFB must be carried through the same entire preparation scheme as the samples including sample digestion, when applicable.
- 8.7.4 The rinse blank is prepared by acidifying reagent water to 2% nitric acid/ 1% hydrochloric acid. (40mL nitric and 20mL hydrochloric into 2L of NANOpure)
- 8.8 Instrument Performance Check (IPC/CCV) Solution - The IPC is a mixed standard obtained from a reputable vendor. . This standard is the same source as the calibration stock standards. The IPC solution is stored in a plastic bottle and analyzed as needed to meet data quality needs (Section 11.1.5). See standard log for recipe.
- 8.9 Quality Control Sample (QCS) – The QCS is a noncalibration source mixed standard containing all certified metals. This standard is used to initially prove method capability (section 14.6.3), demonstrate continuing acceptable instrument performance semiannually via the Performance Testing program (section 14.7) and to verify calibration standards after new standard preparation (section 11.1.6). See standard log for recipe.
- 8.10 Initial Calibration Verification (ICV) – The ICV is a mixed standard obtained from a reputable vendor. This is a noncalibration source standard. The ICV solution is stored in a plastic bottle and analyzed as needed to meet data quality needs. See standard log for recipe.
- 8.11 Spectral Interference Check (SIC) Solution and Blank - When interelement corrections are applied, SIC solutions are needed containing concentrations of the interfering elements at levels that will provide an adequate test of the correction factors. See standard log for preparation information.
- 8.12 Intensity Check (Plasma Solution) - The plasma solution is used for determining the optimum viewing height of the plasma above the work coil prior to using the method. The plasma solution (tuning solution) utilized on the Optima is 10 mg/L

Manganese per manufacturers instruction PE CH 8. The instrument is subjected prior to analysis to the autotune procedure provided with the software.

- 8.13 Internal Standard: A Lanthanum solution is added to all standards and samples. It is added either manually or through on line addition. See standard log for recipes.
- 8.14 A high check is performed with each run to prove linearity. Results must be within 90-110% recovery to accept sample with elevated results.

9.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 9.1 Prior to the collection of an aqueous sample, consideration should be given to the type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. If properly acid preserved, the sample can be held up to 6 months before analysis.
- 9.2 For the determination of the dissolved elements, the sample must be filtered through a 0.45-um pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. (Glass or plastic filtering apparatus are recommended to avoid possible contamination. If the filtered blank shows a problem with the glass apparatus, only plastic apparatus should be used when the determinations of boron and silica are critical. Use a portion of the filtered sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH < 2.
- 9.3 For the determination of total recoverable elements in aqueous samples, samples are not filtered, but acidified with (1+1) nitric acid to pH < 2 (normally, 3 mL of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of collection; however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination it is recommended that the samples be returned to the laboratory within two weeks of collection and acid preserved upon receipt to the laboratory. Acid must be added to the original sampling container. Sample must be in contact with acid for at least 24 hours prior to removing an aliquot from the original container. If analysis begins <24 hours after sample preservation and pH verification, a qualifier will accompany the data.
- 9.4 The pH of all preserved samples is *verified upon receipt by the laboratory*, and if necessary, additional acid is added to bring the pH to <2. If the sample is reactive upon addition of acid, the splitter lets the reaction finish and then rechecks the pH.
- 9.5 A field blank is prepared and analyzed if required by the data user. Use the same container and acid as used in sample collection.
- 9.6 Samples containing turbidity < 1 NTU will be determined by "direct analysis". Screen acidified samples by testing an aliquot in a turbidimeter meter. Results are recorded in the logbook. Samples containing turbidity >1 NTU must be digested prior to analysis.

10.0 QUALITY CONTROL

10.1 Laboratory reagent blank (LRB) or Method Blank (MB)- The laboratory must analyze at least one LRB with each batch of 20 or fewer samples of the same matrix. LRB data are used to assess contamination from the laboratory environment. For batch acceptance, LRB values must be $< \frac{1}{2}$ the reporting limit. If one half the reporting limit is less than the MDL, LRB values must be $<$ the MDL for those analytes. If the LRB is unacceptable, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained. Sample results at or above the reporting limit must be accompanied with a qualifier if the method blank exceeds a concentration greater than $\frac{1}{10}$ the measured raw concentration of the sample. Blank results below the $\frac{1}{2}$ the reporting limit (or below the MDL) are considered to be ND and will not require a note.

10.2 Laboratory fortified blank (LFB) or Blank Spike (BS)- The laboratory analyzes at least one LFB with each batch of samples per matrix type. This check is made from a noncalibration source. Calculate accuracy as percent recovery using the following equation:

$$R = \frac{\text{LFB} - \text{LRB}}{s} \times 100$$

where:

R = percent recovery.

LFB = laboratory fortified blank.

LRB = laboratory reagent blank.

s = concentration equivalent of analyte added to fortify the LBR solution.

If the recovery of any analyte falls outside the required control limits of that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

10.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85-115%. When sufficient internal performance data become available (usually a minimum of twenty to thirty analyses), optional control limits can be developed from the mean percent recovery (\bar{x}) and the standard deviation (S) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \bar{x} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \bar{x} - 3S$$

The optional control limits must be equal to or better than the required control limits of 85-115%. The standard deviation (S) data are used to establish an on-going precision statement for the level of concentrations included in the LFB.

10.4 Laboratory Fortified Matrix (LFM) or Matrix Spike (MS) - Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect.

10.5 The laboratory adds a known amount of each analyte for every 10 routine samples per matrix type per batch. In each case the LFM aliquot is a duplicate of the aliquot used for sample analysis and for total recoverable determinations

added prior to sample preparation. The added analyte concentration is the same as that used in the laboratory fortified blank. See section 8.7.3 for recipe. Samples are chosen at random.

- 10.6 Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 70-130%. Recovery calculations are not required if the concentration added is less than 25% of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C(s) - C}{s} \times 100$$

where:

- R = percent recovery.
C(s) = fortified sample concentration.
C = sample background concentration.
s = concentration equivalent of analyte added to fortify the sample.

- 10.7 If the recovery of any analyte falls outside the designated LFM recovery range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.3), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The data user should be informed that the result for that analyte in the unfortified sample is suspect due to either the heterogeneous nature of the sample or matrix effects.

11.0 PROCEDURE

11.1 Calibration

- 11.1.1 Specific wavelengths are listed in Table 2. Other wavelengths may be substituted if they can provide the needed sensitivity and are corrected for spectral interference. Instrument Operating Conditions: The analyst follows the instructions provided by the instrument manufacturer unless other conditions provide similar or better performance for a task.

- 11.1.1.1 Prior to using this method optimize the plasma operating conditions using the 1 mg/L manganese solution and the autotune program provided with the software.

- 11.1.1.2 Calibrate the instrument per manufacturer's instructions using a single point calibration.

- 11.1.2 Before using the procedure (Section 11.0) to analyze samples, there must be data available documenting initial demonstration of performance. The required data and procedure is described in Section 14.6. This data must be generated using the same instrument operating conditions and calibration routine to be used for sample analysis. These documented data must be kept on file and be available for review by the data user.

- 11.1.3 After completing the initial demonstration of performance, but before analyzing samples, the laboratory must establish and initially verify an interelement spectral interference correction routine to be used during sample analysis. A general description concerning spectral interference and the analytical requirements for background correction and for correction of interelement spectral interference in particular is given in Section 5.1. To determine the appropriate location for background correction and to establish the interelement interference correction routine, repeated spectral scan about the analyte wavelength and repeated analyses of the single element solutions may be required. Criteria for determining an interelement spectral interference is an apparent positive or negative concentration on the analyte at the *MDL level*. Once established, the entire routine must be initially and periodically verified by successful analysis of the SIC blank and check solution with each run, or whenever there is a change in instrument operating conditions.
- 11.1.4 Calibration Blank - A calibration blank is analyzed immediately following daily calibration, after every tenth sample and at the end of the sample run. *Analytes must be < 1/2 the reporting limit*. If one half the reporting limit is less than the MDL, calibration blank values must be < *the MDL* for those analytes.
- 11.1.5 Instrument performance check/ Continuing calibration verification (IPC/CCV) solution - The IPC(CCV) is a solution of metals from the same source as the calibration. The laboratory analyzes the IPC solution following daily calibration, after every tenth sample and at the end of the sample run. Analysis of the IPC solution immediately following calibration must verify that the instrument is within +/- 5% of calibration with a relative standard deviation < 3% from 3 replicate integrations. Subsequent analyses of the IPC solution must be within +/- 10% of calibration. If the calibration cannot be verified within the specified limits, reanalyze either or both the IPC solution and the calibration blank. If the second analysis of the IPC solution or the calibration blank confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined, corrected and/or the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data. Alternately data may be reprocessed using the most recent CCB as the calibrator. Reprocess all samples back thru the last working calibration check. An earlier ICV and CCB reprocessed under the new calibration must work to accept the calibration. All data must be bracketed by working QC. The decision to accept any data when an IPC is out must be clearly documented and made by an authorized member of management.
- 11.1.6 Quality Control Sample (QCS) - The QCS is a solution of metals from a noncalibration source. After the preparation of new calibration standards, analyze three QCS standards. Tabulate the mean concentration of the three QCS analyses. It must be within 5% of the true value to verify the new standards.
- 11.1.7 Initial Calibration Verification (ICV) - The ICV is a solution of metals from a noncalibration source. The ICV is analyzed immediately following initial calibration. Apply the following acceptance criteria:
- 11.1.7.1 The ICV is used to verify initial calibration – acceptance criteria
10% recovery

- 11.1.7.2 If data must be reprocessed under a new calibration due to instrument drift later in the run, a later CCV may be used as the calibrator. The ICV must be processed under the new calibration to verify it - acceptance criteria: 10% recover.
- 11.1.8 Spectral interference check (SIC) solution - The laboratory verifies the interelement spectral interference correction routine by analyzing a SIC solution and blank with each run that contains analytes of interest needing correction. It is analyzed at the beginning, after every 20 samples, and at the end of the run. Target analytes in the SIC solution must be within $\pm 20\%$ of expected value and less than \pm the RL in the SIC blank to verify the correction routine. (If SIC blank is high, see section 10.2 for qualifying criteria.) All interelement spectral correction factors are updated if the SIC solution and SIC blank do not consistently meet acceptance criteria. To update correction factors separate standards of each metal are analyzed at a level of 100ppm (except Fe -300ppm and Al -200ppm). Target metals are examined for any false response caused by the elevated metal.
- 11.2 Aqueous Sample Preparation - Dissolved Analytes
 - 11.2.1 Samples are received, from the splitter, filtered through a 0.45 μ m filter and preserved. The sample is then processed like the samples in paragraph 11.2. The final report indicates that the results are for dissolved analytes.
- 11.3 Preparation for "direct analysis" of total recoverable analytes in samples containing turbidity < 1 NTU – Place in autosampler tube:
 - 11.3.1 5 mL of sample
 - 11.3.2 100 μ Ls conc HNO_3
 - 11.3.3 50 μ L HCl
 - 11.3.4 50 μ L of 2000ppm La Internal Standard.

(If using the FAST system this step may be omitted.)
- 11.4 Total Recoverable Analytes – See SOP for sample preparation.
- 11.5 Sample Analysis
 - 11.5.1 When a problem is suspected, inspect the sample introduction system including the nebulizer, torch, injector tube and uptake tubing for salt deposits, dirt and debris that would restrict solution flow and affect instrument performance. Clean the system when needed or on a daily basis.
 - 11.5.2 Configure the instrument system per manufacturer's instructions.
 - 11.5.3 The instrument must be allowed to become thermally stable before calibration and analyses. This usually requires at least 30 to 60 minutes of operation. After instrument warm-up, complete any required optical profiling or alignment particular to the instrument.
 - 11.5.4 For initial and daily operation calibrate the instrument according to the instrument manufacturer's recommended procedures, using mixed calibration standard solution and the calibration blank. A peristaltic pump is used to introduce all solutions to the nebulizer. To allow equilibrium to be

- reached in the plasma, aspirate all solutions for 30 sec after reaching the plasma before beginning integration of the background corrected signal to accumulate data. When possible, use the average value of replicate integration periods of the signal to be correlated to the analyte concentration. Using the FAST system, flush the line for the equivalent of 60 seconds in between injections. The calibration line should consist of a minimum of a calibration blank and a high standard. Replicates of the blank and highest standard provide an optimal distribution of calibration standards to minimize the confidence band for a straight-line calibration in a response region with uniform variance.
- 11.5.5 After completion of the initial requirements of this method, samples should be analyzed in the same operational manner used in the calibration routine with the rinse blank also being used between all sample solutions, LFBs, LFMs, and check solutions.
- 11.5.6 During the analysis of samples, the laboratory must comply with the required quality control described in Section 10. Only for the determination of dissolved analytes or the "direct analysis" of drinking water with turbidity of < 1 NTU is the sample digestion step of the LRB, LFB, and LFM not required.
- 11.5.7 Any elements with a concentration above the verified linear range must be diluted. Also, for the interelement spectral interference correction routines to remain valid during sample analysis, the interferant concentration must not exceed its verified linear range. In these circumstances analyte detection limits are raised and determination by another approved test procedure that is either more sensitive and/or interference free is recommended.
- 11.5.8 Report data as directed in Sections 11.7-11.16.
- 11.6 Our laboratory uses the internal standard technique (as an alternate to the method of standard addition) by adding one or more elements (not in the samples and verified not to cause an uncorrected interelement spectral interference) at the same concentration (which is sufficient for optimum precision) to the prepared samples (blanks and standards) that are affected the same as the analytes by the sample matrix. The ratio of analyte signal to the internal standard signal is used for calibration and quantitation. This technique is very useful in overcoming matrix interferences especially in high solids matrices. The analyst monitors internal standard response for large drops that might indicate improper spiking of La or sample matrix interference.
- 11.7 Sample data should be reported in units of ug/L for aqueous samples.
- 11.8 For dissolved aqueous analytes report the data generated directly from the instrument with allowance for sample dilution. Do not report analyte concentrations below the MDL.
- 11.9 All data is sent to the LIMS.
- 11.9.1 Aqueous preparation initial and final volumes are 30/15mLs. If an aliquot other than the default volume is used during preparation, this variation is documented in the LIMS initial volume.
- 11.9.2 If a dilution is made at the instrument:

- 11.9.2.1 When using the Fast system, the dilution is entered in LIMS which will also adjust the sample result, MDL, and RL.
- 11.9.2.2 When not using the Fast system, the change in internal standard response will automatically adjust the result prior to data transfer. To correctly report the MDL and RL to reflect this dilution, the transferred result is divided by the dilution factor and entered as raw and the dilution factor is placed in the LIMS DIL column.
- 11.10 The result labeled in the run as "Si" is actually a silica (SiO_2) result and will be entered as such. Silicon standards used to calibrate for silica are entered as silica to account for this conversion.
- 11.11 Total Hardness may be calculated from the calcium and magnesium results as follows (SM 2340 B):

$$\begin{array}{rclcl}
 \text{Ca mg/L} & & \text{Mg mg/L} & & \\
 \text{-----} & + & \text{-----} & \times 50 = & \text{Total Hardness in mg equiv. CaCO}_3/\text{L} \\
 20 & & 12 & &
 \end{array}$$

11.12 Exporting and Entering Data: ICP-OPTIMA 5300DV

- 11.12.1 Delete calculations from QC and the source sample if not requested by the client i.e.: total hardness, total cations, sodium percentage, CaCO_3 , SAR
- 11.12.2 Filtered blanks need to be qualified: QBfil
- 11.12.3 Add qualifier to source sample if the analyte is not requested: ?QC
- 11.12.4 Check for perfect zero - 0 could be saturated sample

11.13 Qualify:

- 11.13.1 Blank: A blank that does not meet the criteria must be qualified QBLK. A blank does not turn red if the result is too negative. The result may not be greater than $\pm \frac{1}{2}$ the RL.
- 11.13.2 BS: A BS that is out of range may be qualified QLMS if the MS meets the BS criteria. If a BS is out of range and can not be qualified the analytes in question must be re-analyzed unless the results are ND and the BS is high.
- 11.13.3 MS:
- 11.13.3.1 If both spikes are red check prep dilution and source sample prep dilution, correct on bench sheet if necessary. Check if source sample is greater than three times the BS value. If it is Qualify the MS: QM-3X
- 11.13.3.2 If all other analytes pass MS criteria and the LCS and CCVs pass for the out analyte, then sample matrix is suspected. Attach QMint to the QC and NMint to the sample and document fully.
- 11.13.3.3 QFnt: If the source sample does not require the reporting of the analyte.
- 11.13.3.4 QOcal if result is over LDR.
- 11.13.4 Verify dilutions on prep sheet and in Lims

11.13.5 Samples, diluted, that result in an answer of ND or J flag: "N_RLm"

11.13.6 Right click Sort by analyte order

11.13.6.1 Check ND results for any negatives greater than RL.

11.13.6.2 If a blank has been qualified, check for any analytes less than 10 times the blank result. This result may be passable if it is ND, if it is not it must be RE'd and Qualified SUS; or qualified NBLK. The result may be reported if it is 10x greater than the blank result. The re's should be addressed now in Project Management, update status. Edit comment, create bench sheets if necessary.

11.13.6.3 Check for results over the LDR, these need to be RE'd , entered as 9999 and qualified ?SUS NOcal

11.13.7 Sort by Sample ID.

11.13.7.1 Calculate any sodium percentage or SAR's

11.14 Fill out peer review sheet

11.15 Water sample results within +/- 10% of the MCL (see table 1) are automatically rechecked.

11.15.1 If the recheck is within 20% RPD* of the original, the result is confirmed and the higher of the 2 results is reported to the client. This is the most conservative approach in the interest of public health.

11.15.2 If the result does not confirm within the RPD criteria, inject the sample a third time to determine the true result.

11.16 *Analytes with MCLs near the Reporting Limit are confirmed if the results are within \pm RL from each other.

12.0 CORRECTIVE ACTION FOR OUT OF CONTROL OR UNACCEPTABLE DATA

12.1 Refer to Technical Corrective Action SOP

13.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

13.1 Refer to "Hazard Communication section of the Chemical Hygiene Plan".

13.2 Waste Disposal

13.2.1 Instrument waste is neutralized and poured down the sink.

13.2.2 Sample waste after digestion is placed in the "High Acid Concentration Waste" barrel.

13.2.3 Samples that are hazardous are marked with a red dot on the original container. An entry is made in the log. Samples are held downstairs for pick up by an independent hazardous waste hauler

14.0 METHOD PERFORMANCE

14.1 The method detection limit (MDL) is the lowest concentration that can be detected for a given analytical method and sample matrix with 99% confidence that the analyte is present. The MDL is determined according to the laboratory's MDL procedure in Appendix H of the QA Manual. MDLs reflect a calculated (statistical)

value determined under ideal laboratory conditions in a clean matrix, and may not be achievable in all environmental matrices. The laboratory maintains MDL studies for analyses performed; these are verified at least annually unless method requirements require a greater frequency.

- 14.2 LOD Verification: On a yearly basis, a QC sample is spiked at a level of not more than 1-4 times analyte MDL. The sample is analyzed. Analyte response must be greater than method blank response to verify the Level of Detection or MDL.
- 14.3 IDOC Initial Demonstration: Prior to initial analysis of samples or when a significant change is made to the method, a Demonstration of Capability Study is performed by each analyst. This is accomplished by analysis of four replicates of a QC sample made at a concentration of 1-4 times the RL. Concentrations of cation metals may be higher. The average percent recovery of the QC samples must be 80-120% with a maximum %RSD of 20. Past studies have been performed at 50-2000ppb.
- 14.4 Demonstration of Continuing Proficiency: On an annual basis, each analyst must turn in valid LCS data from four consecutive batches or results from a successful Performance Evaluation Study. LCS percent recovery must meet laboratory prescribed acceptance criteria. Relative standard deviation between the four replicates must be less than or equal to 20%.
- 14.5 Edward S. Babcock & Sons operates a formal quality control (QC) program. The requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks (LCS's) and other laboratory solutions as a continuing check on performance. The laboratory maintains performance records that define the quality of the data thus generated.
- 14.6 Demonstration of Method Capability.
 - 14.6.1 The Demonstration of Method Capability is used to characterize instrument performance (analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analyses of this method conducted by this laboratory.
 - 14.6.2 Linear dynamic range (LDR) –The upper limit of the linear dynamic range must be established. The LDR should be determined by analyzing succeeding higher standard concentrations of the analyte until the observed value is no more than 10% below the stated concentration of the standard. Analyte concentrations above 90% of the determined upper range are diluted. For those analyses that are known interferences, and are present at above the linear range, the analyst ensures that the interelement correction has been applied correctly. New dynamic ranges should be determined whenever there is a significant change in instrument response. The range is checked regularly. See section 8.14.
 - 14.6.3 Quality control sample (QCS) - When beginning the use of this method, verify calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. The determined mean concentrations from 3 analyses of the QCS must be within +/- 5% of the stated values. If the calibration standard cannot be verified, performance of the determinative step of the method is unacceptable. The source of the

problem must be identified and corrected before either proceeding on with the initial determination of method detection limits.

- 14.6.4 Method detection limit (MDL) – MDL's are established whenever there is a change in instrumentation, or a major modification to the analysis. MDL's must be established for all wavelengths utilized, using reagent water (blank) fortified at a concentration of approximately 2-5 times the estimated detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (S)$$

where:

t = students' t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

S = standard deviation of the replicate analyses. See the attached LIMS information for the current MDL study results.

- 14.6.5 Spectral Interference calculations: The analyst is required to determine and document for each wavelength the effect from known interferences as part of initial instrument set up. See section 5.1.

- 14.7 Internal standards are used with all analyses. Directions for using internal standard(s) are given in Section 11.3.4.

- 14.8 A Detection Limit Check is analyzed with every calibration at the detection limit concentration. 50 – 150% of the expected value is considered acceptable.

15.0 REFERENCES

- 15.1 U.S. Environmental Protection Agency. Inductively Coupled Plasma- Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes- Method 200.7, Supplement 1, Rev 4.4, May 1994
- 15.2 Inductively coupled argon plasma emission spectrometer: Perkin Elmer Optima 5300DV Instrument Manual

16.0 METHOD VARIATIONS

- 16.1 All *italicized items* are an indication of a variation from the method.

17.0 TABLES AND FIGURES

Table 2: Recommended Wavelengths and Detection Limits

Analyte	Wavelength (nm)	Estimated Detection Limit (ug/L)	Calibrate to (mg/L)
Aluminum	308.215	45	10
Antimony	206.833	32	5
Arsenic	193.759	53	10
Barium	493.409	2.3	1
Beryllium	313.042	0.27	1
Boron	249.678	5.7	1
Cadmium	266.502	3.4	2

Calcium	315.887	30	10
Chromium	205.552	6.1	5
Cobalt	228.616	7	2
Copper	324.754	5.4	2
Iron	259.94	6.2	10
Lead	220.353	42	10
Lithium	670.784	3.7	5
Magnesium	279.079	30	10
Manganese	257.61	1.4	2
Molybdenum	203.844	12	10
Nickel	231.604	15	2
Potassium	766.491	700	20
Selenium	196.09	75	5
Silica (SiO ₂)	251.611	26	10
Silver	328.068	7	0.5
Sodium	588.995	29	10
Strontium	421.552	0.77	1
Thallium	190.864	40	5
Tin	189.98	25	4
Titanium	334.941	3.8	10
Vanadium	292.402	7.5	2
Zinc	213.856	1.8	5

Note: The Wavelengths, Detection Limits and Calibration levels listed are recommended because of their sensitivity and overall acceptability. Other values may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference.

Table 3: Analyte Interferences

Analyte	Wave-length	Al	Ba	Be	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Mo	Ni	Ti	Tl	V	Zn
Ag	328.07									x								
Al	396.15				x		x			x			x	x				x
As	188.98	x	x							x	x							
B	249.72						x			x			x					
Ba	233.53																x	
Be	313.11																x	
Cd	228.8						x											
Ca	317.93																	
Cd	228.80																	
Co	228.62		x												x			
Cr	267.72																	
Cu	327.39				x	x	x			x			x		x		x	
Fe	238.2						x				x							
K	766.49																	
Mg	285.21																	
Mn	257.61												x					
Mo	202.03																	
Na	589.59																	
Ni	231.6		x				x											
Pb	220.35	x			x				x									
Sb	206.84				x			x		x			x					
Se	196.03	x			x		x	x		x			x	x	x	x	x	

Tot Si	251.61												x					
Sn	189.93									x					x			
Sr	407.77		x	x						x	x	x		x				
Ti	334.94																	
Tl	190.8							x				x	x	x		x		x
V	310.23	x	x	x	x			x	x		x	x	x			x		x
Zn	206.2	x	x						x		x	x	x	x				

x - This metal interferes with this analyte. Spectral correction must be applied.

APPENDIX E

WECK LABORATORIES, INC. STANDARD OPERATING PROCEDURES

Due to proprietary reasons, Weck Laboratories SOPs are not included.

- *E.coli* analysis is analyzed using P/A Colilert™ and Enumeration by the Quanti-Tray method as described by Standard Methods (SM) 9223. Please refer to Weck Laboratories SOP # MIC003.R09 dated May 2015.
- TSS analysis is analyzed using Non-Filterable Residue by Gravimetric Method as described by SM 2540D. Please refer to Weck Laboratories SOP # WET042.R09 dated January 2015.

APPENDIX F

ENTHALPY ANALYTICAL STANDARD OPERATING PROCEDURES

Due to proprietary reasons, Enthalpy Analytical (formerly Associated Laboratories) SOPs are not included.

- *E.coli* analysis is analyzed using the multiple tube fermentation technique (MTF) as described by Standard Methods (SM) 9221. Please refer to Enthalpy Analytical Document # E-0004 dated September 2015.
- TSS analysis is analyzed using EPA Method 160.2 or SM 2540D. Please refer to Enthalpy Analytical Document # D-0005 dated April 2015.

APPENDIX G

WESTON SOLUTIONS STANDARD OPERATING PROCEDURES

Due to proprietary reasons, Weston Solutions SOPs for analytical methods are not included. Supplemental methodology information provided by Weston Solutions is listed as follows:

- Silkie, S.S. and Nelson, K.L., Concentrations of host-specific and generic fecal markers measured by quantitative PCR in raw sewage and fresh animal feces, Water Research 43 (2009) 4860-4871

Title: Filtration Protocol for Samples for Molecular Analysis

SOP No.: LAB074.01

Date: 5/15/15

Page No.: 1 of 4

Prepared By:

Approved By:

Date:

QA Concurrence:

Date:

Kelly Goodin

A. S. Sneider

5/22/2015

A. S. Sneider

5/22/2015

1.0 SCOPE

This document describes general procedures for filtering samples for molecular analysis. This protocol applies to a variety of sample types including: fresh water, marine water, and non-liquid matrices “extracted” into liquid (for example, sand, wrack, eelgrass extracted into PBS).

2.0 HEALTH AND SAFETY

2.1 Personnel

Only properly trained technicians will perform the procedures described herein.

2.2 Clothing

All participating personnel must wear disposable gloves, close-toed shoes and any required safety gear for molecular biology laboratory work. Goggles and face masks are worn as appropriate or required. Gloves are changed as per the protocol being followed, after any spills, and when any possible contamination is suspected.

2.3 Practices

- Mouth pipetting is prohibited.
- Bleach is caustic. Wear appropriate personal protection. During procedures involving bleach, wear lab coats, protective glasses, and gloves. Change gloves after bleach application, taking care to not leave bleach on surfaces that others may contact with bare skin (e.g., phone, door knobs, etc.). Remove residual bleach with an ethanol or DI water rinse.
- Do not mix bleach and ammonia-based cleansers. Mixing bleach and ammonia can create toxic chloramine gases and an explosive (nitrogen trichloride).
- Ensure that room is properly ventilated and avoid scented bleaches which may disguise bleach scent.
- Change gloves regularly and when overtly contaminated. Remove gloves before handling door knobs, phones, etc.
- Gloves contaminated or potentially contaminated should be removed with standard procedures for removing contaminated gloves, as follows:
 - Pull one glove near your wrist towards your fingertips until the glove folds over.
 - Carefully grab the fold and pull toward your fingertips, turning the glove inside out as you pull.
 - Remove your hand from the glove, continuing to hold it in the other, gloved hand.
 - Slide a finger from the glove-free hand *under* the remaining glove and remove the glove so that it turns *inside out and encases the other glove*. Place gloves in waste bag.

Title: Filtration Protocol for Samples for Molecular Analysis

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A. S. Sneider

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A. S. Sneider

5/22/2015

- **Take care when handling flammable liquids, as follows:**

- Flammable liquids are to be kept in glass, not plastic.
- Maintain adequate distance between open flame and ethanol.
- When holding flaming forceps, keep hand above the forceps to avoid flaming ethanol dripping onto hand.
- Keep the forceps fairly still until flame has burned out. In particular, do not move flaming forceps over ethanol container. If a drop of flaming ethanol drops into the container, it will ignite.

2.4 Equipment and Materials

- All personnel are responsible to know how to safely utilize/handle all pertinent equipment and materials. Some reagents are potentially harmful. DNA Away and dilute bleach solutions can irritate eyes, skin, and mucous membranes. See MSDS for more information. Copies of Material Safety Data Sheets are available in the Molecular lab, and from the safety officer.

2.5 Waste and Sterilization

- For disinfection, wipe surfaces with a freshly made “10% bleach solution” (bleach:water: 1:9 for ~6% household bleach; 1:15 for 8.25% household bleach) or purchased bleach product (e.g., Clorox Healthcare Bleach Germicidal Cleaners) - See LAB079.00 for more information. Let bleach stand for 15 minutes. Use UV light if available (15 minutes). Change gloves. Rinse metal and surfaces that will contact skin or clothing with sterile DI water after bleaching to remove residual bleach. Spray surfaces with 70% ethanol.
- Bleach is not appropriate for all equipment due to corrosion. Check manufacturers recommendations if unsure. Change gloves after decontamination.
- Biological waste is to be autoclaved and discarded in appropriate autoclave bags according to standard microbiological procedures and local and state rules. Samples, reference materials, and equipment known or suspected to have viable bacteria attached or contained will be sterilized prior to disposal.
- All sharps and glass are disposed of in designated sharps disposal containers.

3.0 Equipment, Materials, and Supplies

- Sterile, disposable membrane filtration units consisting of 100 mL capacity funnel filter base with 47 mm diameter membrane filters (e.g., Pall MicroFunnels™ funnels Supor® Membrane (0.22 µm), part number 4806 or equivalent). Polycarbonate filters (0.45 µm) can also be used.
- Replacement filters to fit funnel, if replicate samples are being processed.
- GeneRite DNA Extraction Kit Pre-loaded bead tubes Part # S205-080.
- Sterile Phosphate Buffered Saline (PBS), 1X

Title: Filtration Protocol for Samples for Molecular Analysis

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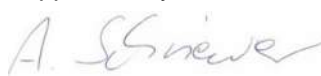
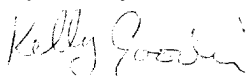
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- Bleach and bleach solution
- Ethanol

4.0 SAMPLE FILTRATION

- 1) Treat work surfaces with a freshly made “10% bleach solution” (bleach water: 1:9 for ~6% household bleach; 1:15 for 8.25% household bleach or purchased bleach product (e.g., Clorox Healthcare Bleach Germicidal Cleaner or equivalent) for 15 minutes, rinse by spraying with 70% ethanol to remove residual bleach (if surface will corrode, rinse with sterile water prior to ethanol treatment). See LAB079.00 for more information.
- 2) Decontaminate filter manifold with DNA AWAY or 10% Bleach / 70% ethanol rinse (use a DI rinse in between if manifold is metal to avoid corrosion). [Although the manifold does not come in direct contact with the samples, decontamination of the manifold reduces the risk of glove contamination and thus reduces the risk of cross-contamination overall].
- 3) Change gloves to avoid spread of bleach.
- 4) Aseptically remove sterile, disposable filter funnels (see Section 3.0) from packaging and place onto the vacuum manifold. Label funnel with sample name.
- 5) To ensure cleanliness in the filtration area, unpack and wipe the exterior of bottles in an area separate from the immediate filtration area that has been sanitized. Check bottles against the Chain of Custody (COC), the Receipt and Process Log. Organize bottles in the order that appears on the COC/Receipt and Process Log, *accounting for the required method blanks*. Immediately contact appropriate staff regarding any inconsistencies. Fill out the Sample Filtration Form.
- 6) Prior to filtering, label GeneRite DNA EZ kit extraction tubes (containing glass beads) for every sample *and method blank* on the top and side and place the tubes in a cleaned (bleached or DNA AWAY) and dried rack, leaving space between tubes in order to minimize the risk of cross contamination between samples. Alternatively, 1.7 mL centrifuge tubes (sterile, nuclease free) can be used, but extraction tubes are preferable.
- 7) A method blank is sterile PBS replacing the environmental sample (typically filter 25 mL, with no rinse; however, definitions vary by protocol. Consult the AssayTechInfo.xls document). All other processes are the same as a sample. Typically 6 - 17 samples will comprise an extraction set (samples extracted at one time). *In general, a set of 3 blanks is needed per extraction set, typically made up of a field blank, a method blank, and an extraction blank; therefore, at least 1 method blank per set of extractions will need to be created. Consult the Project Overview and Receipt and Process Log to ensure that the proper number of method blanks are created, distributed through the sample set, and processed in accordance with the log.*

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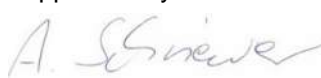
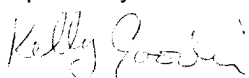
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- 8) To clean the exterior surface of bottles, note the bottle name and wipe the exterior surface with a Kimwipe sprayed with DNA AWAY. Re-label the bottle if needed. Set bottle onto a clean Kimwipe. Repeat, for each bottle using a new Kimwipe for each, and wipe gloves with DNA AWAY between dealing with bottles from a new site. Change gloves when done cleaning the bottles. Bottles are now ready for transfer to the filtration area.
- 9) Immediately before filtering, shake water sample 25 times in an arc motion and pipet 100mL into the funnel using a sterile pipettor. Leaving the funnel lid in place, turn the vacuum pump on and open the vacuum line. After the sample has flowed through just to dryness, close the vacuum line. NOTE: if the sample is visibly sandy or otherwise adulterated, let the sample settle for approximately 1 minute after shaking to minimize filter clogging.
- 10) Rinse sides of funnel with 20-30 mL of sterile PBS and continue filtration until all liquid has been pulled through. Close the valves on the individual manifolds, and turn off vacuum.
- 11) Record observations on the sample filtration form (e.g., filter color). If sample clogs, measure the volume that did not go through and record the actual volume filtered.
- 12) Decontaminate two forceps by dipping both sequentially into small beakers of: 1) fresh bleach, 2) water, 3) 95% ethanol. Flame forceps (see safety precautions). NOTE: the beakers only need a few mLs of liquid, enough to cover the part of the forceps that touches the filter.
- 13) For each filter, remove funnel from the filter base. Leaving the filter on the base, use the forceps to aseptically roll the filter into a cylinder, being careful to only touch the rim of the filter paper that did not contact the sample. NOTE: the same funnel can be used for replicate samples; aseptically replace the filter.
- 14) Insert the rolled filter into the labeled extraction tubes from the GeneRite kit (as prepared in above in step 3.2). Cap tightly. Freeze tubes at -80°C. Flash freezing is best; if possible, dip in liquid nitrogen or a dry ice/ethanol slurry before transfer to -80°C – but ensure that tubes are capped tightly.

Title: GeneRite DNA-EZ Extraction (500/350) with Freezing Filter Dry

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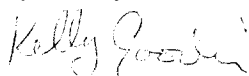
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1.0 SCOPE

This document describes DNA extraction from filtered samples using the GeneRite DNA-EZ kit with filters frozen dry in GeneRite extraction tubes and using 500 μ L lysis buffer to 350 μ L clarified lysate for extraction (1.43 correction factor), as per the **method of DNA extraction used in the Source Identification Protocol Project (SIPP)**.

Note that a variety of protocols utilize the GeneRite DNA-EZ kit modified from the manufacturer's instructions. Common differences among protocols include the volume of lysis buffer added, the volume of crude lysate transferred away from the glass beads, the volume of clarified lysate transferred, whether the elution buffer is warmed, and bead beat and centrifuge times at the various steps. In addition, not all calculation protocols utilize the correction factor (lysis buffer volume/clarified lysate volume) to correct for DNA lost by not carrying over the full lysis buffer volume. **Check the project overview form and the process log for requested deviations from this SOP.** If in doubt consult the technical manager or responsible project manager before performing the DNA Extraction. Otherwise you risk losing an entire batch of samples that cannot be replaced.

2.0 HEALTH & SAFETY

2.1 Personnel

Only properly trained technicians will perform the procedures described herein.

2.2 Clothing

All participating personnel must wear disposable gloves, close-toed shoes and any required safety gear for molecular biology laboratory work. Goggles and face masks are worn when appropriate or required. Gloves are changed as per the protocol to follow, after any spills and when any possible contamination is suspected.

2.3 Practices

During procedures involving bleach, wear lab coats, protective glasses, and gloves. Change gloves after bleach application, taking care to not leave bleach on surfaces that others may contact with bare skin (e.g., phone, door knobs, etc.). Remove residual bleach with an ethanol or DI water rinse.

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Kelly Goodin

A. S. Sneider

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A. S. Sneider

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2.4 Equipment, Materials, and Conditions

All personnel are responsible to know how to safely utilize/handle all pertinent equipment and materials. DNA-Away and dilute bleach solutions can irritate eyes, skin, and mucous membranes. See MSDS for more information.

All personnel present in the lab must wear proper hearing protection when the Bead Beater is running.

3.0 EQUIPMENT AND SUPPLIES

3.1 Equipment

Dedicated DNA Hood for extractions, equipped with UV light
Centrifuge in DNA Hood
Bead Beater
Set of Calibrated Micropipettors (20 μ L, 200 μ L and 1000 μ L)
-80°C Freezer

3.2 Supplies

GeneRite DNA-EZ ST1 Kit, P/N K200-01C-50 or equivalent
1.7-ml low-retention plastic microtubes (autoclaved & dried)
Low-retention, filtered micropipette tips (1000 μ L and 200 μ L)
disposable laboratory gloves
nuclease-free water
DNA-Away®
Kimwipes (large)
water-proof, felt-tip markers
70% Ethanol
10% Bleach

4.0 PROCEDURES

4.1 Before Starting

Spray work surfaces with 10% bleach solution, let sit for ~ 10-15 minutes and spray with 70% ethanol. Wipe dry if needed. For metal and contact surfaces (surfaces that can contact skin and clothing), rinse with sterile DI prior to ethanol.

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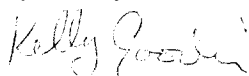
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4.2 Preparing Lysis Buffer Containing Sample Process Control (SPC)

As default salmon sperm DNA (sketa) is used as an extraction process control. It is added with the lysis buffer in a concentration of 0.2 µg salmon sperm per mL of lysis buffer. The sketa/lysis buffer mixture is prepared daily prior to extraction. It is mandatory to create a batch record each time and to enter the batch ID into the DNA extraction form.

4.3 Extraction Procedure

- 1) Remove sample/filter extraction tubes from the freezer.
- 2) Add 500 µL of sketa/lysis buffer mix to each extraction tube. Pipetting slowly reduces foaming.
- 3) Make sure that tubes are also labeled on the side, as the bead beater can remove writing from the top of the tubes.
- 4) Warm elution buffer by adding sufficient volume (plan on 100 µL per sample, plus extra for pipetting error) to sterile centrifuge tubes and place in heating block preheated to 60°C.
- 5) Label needed tubes for each sample. Label side and tops. Label an additional set of tubes for a method blank – see below. *(1.5mL tubes can be used, but easier with 1.7mL).*
- 6) To one of the tubes for each sample, add 1mL of binding buffer.
- 7) Check that samples are closed tightly and bead beat for **2 minutes** at maximum speed.
- 8) Centrifuge for **1 minute** at 12,000 rcf.
- 9) Place tubes into rack. *Throughout this extraction leave space between and only have one tube open at a time to minimize the chance of contamination.*
- 10) **Transfer the maximum amount** of crude supernatant to a corresponding labeled sterile microcentrifuge tube, using a 200 µL pipette two times. Record volume obtained if less than 350 µL. *Tilt the tube to get maximum volume and avoid the pellet.*
- 11) Centrifuge for **1 min** at 12,000 rcf.
- 12) Without disturbing the pellet, transfer **350 µL** clarified supernatant to the labeled tube containing 1.0 mL binding buffer solution. **Record volume if less than 350 µL.**
- 13) Thoroughly mix the sample and binding buffer by gently vortexing (can also mix by pipetting up and down).
- 14) Pulse fuge to get the liquid off the cap.
- 15) Insert a DNAsure column into each collection tube if not already combined. **Transfer 700 µL** binding buffer/extract solution to each tube.
- 16) Centrifuge for 1 min at 10,000 rcf.
- 17) Transfer each column to a new collection tube. Discard old tube and liquid.
- 18) **Transfer the remaining** binding buffer/extract solution to the corresponding spin column in each new collection tube.

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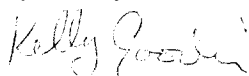
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- 19) Centrifuge for 1 min at 10,000 rcf.
- 20) Transfer each column to a new labeled collection tube. Discard old tube and liquid.
- 21) Add **500 µL** of the EZ-Wash Buffer.
- 22) Centrifuge for 1 minute at 10,000 rcf. Discard flow through (Optional: change the collection tube).
- 23) Repeat by adding **another 500 µL** Wash Buffer.
- 24) Centrifuge **1 minute** at 10,000 rcf. Discard flow through.
- 25) After washing the second time, place column into new collection tube and centrifuge for **1 minute** at 10,000 rcf in order to remove any residual ethanol.
- 26) **Transfer** each column to the final labeled low-retention microcentrifuge tube.
- 27) Add **50 µL** of warm DNA elution buffer to each column. *Pipet directly into the middle of the column.*
- 28) Allow the buffer to sit on the filter for **1 minute**.
- 29) Centrifuge for **1 minute** at 10,000 rcf. **KEEP FLOW THROUGH.** This is your DNA!
- 30) **Repeat** with another **50 µL** of elution buffer, **sit for 1 minute, centrifuge 1 minute** at 10,000 rcf. For a total of 100 µL of purified DNA.
- 31) Vortex/pulse fuge the DNA.
- 32) Aliquot 50 µL (or less) to clean 0.6 mL low retention tubes and archive in a labeled box at -80°C (or -20°C not frost-free for short term). How many aliquots will depend on how many assays will be run with that DNA (avoid freeze-thaw).
- 33) If using within a week, store an aliquot at 4°C until use rather than freezing.
- 34) If required, take Nanodrop measurements for each purified DNA sample at least in duplicate against an elution buffer blank. Record A260 ratios.

4.4 Method and Extraction Blanks

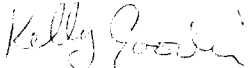


Consult LAB074.01 and LAB073.01 for information regarding method and extraction blanks.

5.0 QUALITY CONTROL/ASSURANCE

All participating personnel are fully trained in the techniques of handling and processing samples for molecular analysis as well as the use of any specialized equipment required to perform above procedures.

Precautions are taken to avoid exposing samples to human, atmospheric, and other potential sources of contamination.

Disposable gloves are worn at all times when handling samples, supplies and equipment used throughout these procedures. If gloved hands contact any surface that is suspected of being a contamination risk, the glove is removed and replaced with a fresh, clean one.

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		5/26/2015		5.26/2015

6.0 REFERENCE DOCUMENTS

Boehm, A.B., Van De Werfhorst, L.C., Griffith, J.G., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D.W., Weisberg, S.B. 2013. Performance of Forty-Three Microbial Source Tracking Methods: A Twenty-Seven Lab Evaluation Study. Water Research 47(18) 6812-6828, <http://dx.doi.org/10.1016/j.watres.2012.12.046>.

DNA EZ (RW03) extraction SOP for SIPP (SIPP GeneRite DNA-EZ Extraction Protocol). SCCWRP. 2012

Schriewer, A., Goodwin, K.D., Sinigallinao, C.D., Cox, A., Wanless, D., Bartkowiak, J., Ebentier, D.L., Hanley, K.T., Ervin, J., Deering, L.A., Shanks, O.C., Peed, L.A., Meijer, W.G., Griffith, J., Santo Domingo, J., Jay, J.A., Holden, P.A., and Wuertz, S. 2013. Performance Evaluation of Canine-Associated Bacteroidales Assays in a Multi-Laboratory Comparison Study. Water Research, 47(18), 6909-6920, <http://dx.doi.org/10.1016/j.watres.2013.03.062> (2013).

Sinigalliano, C.D., Ervin, J., Van de Werfhorst, L.C., Badgley, B.D., Balleste, E., Bartkowiak, J., Boehm, A.B., Byappanahalli, M., Goodwin, K.D., Gourmelon M., Griffith, J., Holden, N.M., Jay, J., Layton, B., Lee, C., Lee, J., Meijer, W.G., Noble, R., Raith, M., Ryu, H., Sadowsky, M.J., Schriewer, A., Wang, D., Wanless, D., Whitman, R., Wuertz, S., and Santo Domingo, J. 2013. Multi-Laboratory Evaluations of the Performance of *Catelicoccus marimammalium* PCR Assays Developed to Target Gull Fecal Sources. Water Research 47(18), 6883-6896, <http://dx.doi.org/10.1016/j.watres.2013.02.059>.

US EPA. Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay, October 2012. EPA-821-R-12-008.

US EPA. Method 1611.1: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay, April 2015. EPA-820-R-15-008.

APPENDIX H

SOURCE MOLECULAR STANDARD OPERATING PROCEDURES

Due to proprietary reasons, Source Molecular SOPs for analytical methods are not included. Relevant Source Molecular SOPs are listed as follows:

- Test Method 1 – Preparation of Water, Environmental and Biological Samples (v.1)
- Test Method 2 – Nucleic Acid Extraction (v.2)
- Test Method 3 – QPCR for microbial source tracking (v.4)

Sampling and Packing Instructions

Sample Shipping Kit Contents

- Plastic Cooler
- 500 mL Sterile Sampling Bottles
- Zip Bags for Bottles
- Ice Packs
- Packing Tape
- Chain of Custody Form

Instructions

Prior to Sample Collection

1. Place ice packs flat in freezer upon receiving the sample shipping kit. Please keep ice packs in the zip bags that they arrive in at all times.

Sample Collection

2. Sample water following standard sampling QA measures.
 - a. Wash hands and put on clean disposable gloves.
 - b. Keep bottles closed until just prior to sample collection.
 - c. Be careful not to touch inside of bottle or cap.
3. Properly label each bottle with a permanent marker (ie. Sharpie).
4. Wrap each bottle with abundant paper towels and place each in an individual zip bag.

After Sample Collection

5. If possible, pre-chill the samples prior to shipment. Samples can be pre-chilled in a separate cooler filled with ice.
6. Place bottles upright in cooler with frozen ice packs between them. Place excess ice packs on top of the bottles.
7. Fill out the Chain of Custody Record form, place in zip bag and include in the cooler.
8. Close cooler, tape it shut and tape around the lid to form a seal.
9. Ship cooler via **overnight courier** and email a tracking number along with your contact information to info@sourcemolecular.com. The laboratory will contact you upon receipt of the samples.

Shipping Address

Attention: Thierry Tamers
Source Molecular
4985 SW 74th Court
Miami, FL 33155
(786) 220-0379

Source Molecular QA/QC Summary

Special Training/Certification

Individuals appointed to MST projects hold at minimum a Bachelor's degree and have a sound knowledge in genetics and molecular biology. Individuals must have had 1 year of previous hands-on qPCR experience at another laboratory. Trainees undergo supervised hands-on training by the Laboratory Manager, which typically lasts 1-4 months depending on experience. An initial demonstration of technical capability is required before personnel are permitted to work independently on client projects. This involves:

- Successfully preparing five, 5-point standard curves that satisfy accuracy and precision criteria; and
- A side-by-side comparison test in which the trainee's results are compared to a qualified individual's results after both independently prepare and analyze the same randomly selected client samples (5 batches up to 100 samples).

Training records are documented by the Laboratory Manager and hard copies are kept on file in the company's office.

Quality Objectives and Criteria

Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. Key performance acceptance criteria and Data Quality Indicators are described below.

Data Quality Indicators and QC Requirements for MST Tests

Data Quality Indicators	QC Item/Activity Used to Assess Measurement Performance	Purpose	Frequency	Measurement Performance Criteria
Accuracy/Bias	Extraction blank	Evaluates contamination during DNA extraction/purification	Once every week samples are extracted	No detection or detection at least 3 C_T units above sample C_T values
Accuracy/bias	Diluted sample	Monitors for sample matrix inhibition affects	Every sample analyzed	C_T value must be greater than that of unknown sample
Accuracy/bias	Positive control	Monitors for false negatives	One reaction for every sample analyzed	C_T value below 35. No false negatives

Accuracy/bias	Negative control	Monitors for false positives	Three reactions for every sample analyzed	No detection or detection at least 3 C_T units above sample C_T values
Accuracy/bias	Standard Curve	Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range to accurately quantify samples	One curve in duplicate for every sample analyzed and requiring quantification	R^2 : ≥ 0.98 Efficiency: 80-110% Slope: -3.0 - -4.0 Analytical Limit of Quantification (copies). Sample unknown within the linear dynamic range limits
Precision/Comparability	qPCR duplicates	Ensures precision and confidence in data	Every sample analyzed	± 1 standard deviation unless CT value ≥ 33

Analytical Methods

Test methods meet the needs of the project and are appropriate for the tests undertaken. The microbial source tracking tests aim to identify potential animal host sources of fecal contamination in water samples. Currently, no standard methods exist for microbial source tracking. Genetic markers used for microbial source tracking tests are adopted by the Source Molecular laboratory from published, peer-reviewed scientific texts or journals whenever possible. Tests have been validated internally and/or externally in the microbial source tracking research community. If possible, reference methods published as international, national or regional standards are used. The laboratory ensures that the latest edition of a standard is used unless it is not appropriate or possible to do so.

Quality Control

Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. All QC criteria must be met for the results to be considered valid and reported to client.

Statistical calculations are calculated automatically by the qPCR software. These include qPCR replicate standard deviations, replicate means and standard curve efficiency, slope, y-intercept and coefficient of linear regression (R^2).

QC Item/Activity	Data Quality Indicator	Frequency
Extraction blank	Accuracy/Bias - Evaluates contamination during DNA extraction/purification	Once every week samples are extracted
qPCR duplicates	Precision, Comparability -ensures precision and confidence in data	Every sample analyzed
Diluted sample	Accuracy/Bias -Monitors for sample matrix inhibition affects	Every sample analyzed
Positive control	Accuracy/Bias -Monitors for false negatives	One reaction for every sample analyzed
Negative control	Accuracy/Bias -Monitors for false positives	Three reactions for every sample analyzed
Standard Curve	Accuracy/Bias, Comparability, Sensitivity -Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range to accurately quantify samples	One curve in duplicate for every sample analyzed and requiring quantification

Instrument/Equipment Testing, Inspection, and Maintenance

Access to laboratory equipment is controlled to ensure that only authorized personnel use the equipment. Instructions on the use and maintenance of equipment are readily accessible by authorized personnel.

Generally, the handling, transport, storage, use and maintenance of equipment are outlined in the manufacturer's manual. Manuals are located in the laboratory at all times. Specific requirements, if any, are outlined in the test method standard operating procedures.

The manufacturer's manual is critical in describing the safe handling requirements of the equipment, to avoid any damage, alteration, contamination, deterioration, change of integrity or reliability and condition of the equipment (or samples). The manufacturer's manual also provides guidance for suitable environmental conditions for the calibrations, inspections, measurements and tests performed. These guidelines should be followed at all times unless specified otherwise in standard operating procedures.

Routine test work is completely discontinued on equipment that shows minor non-conformances. Not only do we do this for ethical reasons in support of our customer, but minor non-conformances are often indicative of major breakdowns in expensive equipment. These breakdowns need to be avoided wherever possible. Out of service equipment is clearly marked with an "out of service" label.

General Equipment

General service equipment is maintained by cleaning and performing safety checks as necessary. Calibrations or performance checks will be necessary where the setting can significantly affect the test or analytical result (e.g., the temperature of a water bath). Instructions on the use and maintenance of general equipment are located in the laboratory at all times.

Volumetric Equipment

The correct use of volumetric equipment is critical to analytical measurements. Volumetric equipment are suitably maintained and calibrated as specified in the Equipment Records and Inventory datasheet located in the web-based storage system.

Attention is paid to the possibility of contamination arising from the equipment or cross-contamination from previous use. The type used, cleaning, storage and segregation of volumetric equipment are critical. Volumetric equipment should be sterilized with 10% bleach solution and 70% ethanol, DNA Away, or autoclaved as appropriate. Instructions on the use and maintenance of volumetric equipment are located in the laboratory at all times.

Measuring Equipment

Measuring equipment, which include the real-time qPCR instrument, must be used correctly, with care and requires stringent calibration and maintenance plans to ensure adequate performance. Such equipment shall not be used for measurement of customer test items if they go out of calibration. If this occurs, items must be re-measurement once the equipment has been re-calibrated. Operating instructions for the Applied Biosystems StepOnePlus Real-Time qPCR System are located in the laboratory office and also in the StepOnePlus Software Instrument Maintenance Manager.

Instrument/Equipment Calibration and Frequency

All measurement and test equipment having an effect on the accuracy or validity of tests are calibrated and/or verified before being put into service. Calibration records for these equipment, including calibration dates and due dates, are maintained in the Source Molecular web-based storage system. Equipment may be calibrated internally or externally. External calibration

services must be conducted by a calibration laboratory that demonstrates competence by being accredited and demonstrating measurement capability and traceability. The frequency of calibration depends on the accuracy requirements of the test, the stability of the instrument and manufacturer recommendations. It is crucial that calibration measurements are traceable to the International System of Units (SI) whenever possible. Calibration reports, traceability certificates and certificates of analysis are maintained in the Calibration Certificates binder that is kept in the laboratory office. Records are retained for 5 years or more at the discretion of the laboratory.

The procedures for checking newly received equipment are as determined by manufacturers' specification and/or those determined by the laboratory during procurement.

After repair, equipment must be calibrated, when appropriate, and verified to perform correctly by following procedures in the manufacturer's manual and/or by comparing pre-nonconformance and post-repair tests.

Anytime the equipment goes outside the direct control of the laboratory, the function and calibration status must be verified before the equipment can be returned to service. This is done by ensuring that calibration stickers and calibration reports are correct, calibration values are within a specified range (if applicable) and that all components of the instrument are functioning properly. This, along with other key information, is recorded and documentation is stored for 5 years or more at the discretion of the laboratory. When verification of the calibration status and functionality of the equipment is not possible, the equipment must be re-calibrated and serviced, respectively.

Generally, spare parts do not have to be kept on hand in the laboratory. Any parts that are needed as part of equipment servicing are provided and installed by the manufacturer or service contractor.

Inspection/Acceptance of Supplies and Consumables

For all test methods, only services and supplies of the required quality and grade are used. If the specified reagent or material is discontinued by the manufacturer, an alternative from a different manufacturer may be purchased as long as the grade and specifications are identical to the discontinued item. The Laboratory Manager verifies and approves the alternate items and the change is made in the appropriate SOP. Supplies, materials and consumables to be purchased are determined by the Laboratory Manager and entered into an electronic "order list" that includes a description of the item, the name of the vendor, the item catalogue number, the quantity and the cost and is stored for a minimum of 5 years.

Shipments are received at the receiving area and brought to the laboratory. The Laboratory Manager or other authorized personnel is responsible for checking shipments for accuracy. Packing slips are checked against package content labels and matched with the electronic order list. Certificates of analysis (COA) are verified (when applicable) to ensure the received item

meets minimum specifications. All standards, reagents, filters, and other consumable supplies are purchased from manufacturers with performance guarantees and industry recognition, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality. Reagents are marked with the “date received”. Primers and plasmid DNA standards are quantitated and aliquoted for storage at -80°C.

Once the materials are verified, the appropriate box is checked next to the item in the order list and an electronic signature is created. If a discrepancy is found that could affect the quality of laboratory output, the supplier is contacted and the material is replaced.

All supplies will be stored as per manufacturer labeling and discarded past expiration date. Long term storage of nucleic acids is in a -80°C freezer. Whenever possible, consumables and reagents that come into contact with test samples are received pre-sterilized and disposable (e.g. filtering funnels). They are used once and not re-used. Specific information of supply and consumable vendors are specified in individual Test Method SOPs’ materials list.